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Research Article

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Antifungal Efficacy of Plant Essentail Oils against Eggplant Grey Mould Disease

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Abstract Gray mold caused by *Botrytis cinerea* Pers. causes significant yield losses in many vegetables and fruits. Synthetic chemicals used against the disease negatively effect human health and the environment. Plant essential oils (PEOs) have the potential to replace synthetic chemicals in the management of fruits and vegetables. The aim of this study is to evaluate the contact phase effect of different PEOs (rosemary, black cumin, cumin, sandalwood and ginger) against *B. cinerea*, under *in vitro* conditions. Five PEOs were each tested at six concentrations (0, 200, 400, 600, 800 and 1000 µL L^{-1}). The experiment was carried out in a randomized plot design with three replications. In addition, PEOs were analyzed by Gas Chromatography and Mass Spectrometry (GC-MS). Contact phase effect of different concentrations of the PEOs was found to inhibit the growth of *B. cinerea* in a dose dependent manner. *In vitro* experiments indicated that *B. cinerea* did show mycelium growth in presence of all PEOs at five concentrations. The highest inhibitory effect against *B. cinerea* was detected in the high dose (1000 µL L^{-1}) application of cumin PEO, 62.5%. Cumin PEO has a high antifungal effect against the pathogen because it contains Cuminaldehyde chemical compound. The lowest antifungal effect against pathogen was found in the high dose treatmet of sandalwood PEO, 47.4%. These results have shown that the PEOs derived from cumin might be used as alternative options for the control of eggplant gray mould disease.

Keywords Essential oils, *Botrytis cinerea*, Antifungal activity, Contact phase effect, Alternative control

1. Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable species in the Solanaceae family, widely grown in tropical and subtropical ecologies and the Mediterranean Basin [1]. Eggplant contains many vitamins and minerals such as B1, B6, folate, copper, manganese and potassium, as well as "nasunin", a powerful antioxidant and free radical scavenger [2]. Eggplant is produced economically in both field and greenhouse areas in the Mediterranean, Aegean, Marmara, Black Sea and Southeastern Anatolia Regions of Turkey. According to 2020 data, approximately 56.6 million tons of eggplants are produced in the world. Turkey ranks fourth in the world after China, India and Egypt with approximately 832 thousand tons of eggplant production [3].

Wilt, root rot, gray mold, white rot and powdery mildew diseases cause economic yield losses in eggplant production. *Botrytis cinerea* Pers.: Fr. [telemorph: *Botryotinia fuckeliana* (de Bary) Whetz.] is a parasitic and saprophytic polyphagous fungus with a high ability to adapt to different climate and soil conditions and a wide host range [4]. *B. cinerea* affects more than 200 plant hosts, including apple and strawberry, and is widely spread in various regions [5]. Gray mold is a weakness pathogen. Conidia (asexual spores) of *B. cinerea* are easily spread by wind or water [6]. The fungus commonly causes symptoms such as flower blight, fruit rot, stem and branch rot, leaf spots, root rot and soft rot in infected plants. *B. cinerea* overwinters by forming sclerotia in

soil and plant residues [7]. The annual economic losses of gray mold disease are in the range of tens to hundreds of billion dollars worldwide [8]. *B. cinerea* causes 70-90% yield losses in vineyard production [9], 30-35% yield losses in tomato production [10], 10-70% yield losses in the harvest and post-harvest periods of fruits such as kiwi and watermelon [11] and 20-30% yield losses in eggplants production [12]. As a result, annual economic losses of gray mold disease are approximately 1 billion Euros [13].

Cultural control, resistant varieties, soil disinfection and chemical control are recommended in the control against gray mold disease. Various fungicides such as hydroxyyanilides (fenhexamide), anilinopyrimidines (cyprodinil), dicarboxamides (iprodione), carboxamides (boscalid), strobilurins, phenylpyrroles (fludioxonil) are widely used in chemical control against gray mold disease [14]. Despite fungicide spraying, pathogen infections can not be prevented. The use of synthetic fungicides causes residues in food, toxicity to non-target organisms, formation of resistance and environmental problems [15]. Therefore, there is a need for alternative environmentally friendly control methods to chemical control [16]. Natural products are readily available, environmentally safe, have a low risk of developing resistance to pests, and have fewer negative effects on animals [17].

Essential oils (EOs) are one of the most promising groups of natural compounds for the development of antimicrobial agents and their use in plant protection. In general, EOs are complex mixtures of hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes, and related compounds derived from the secondary metabolism of plants [18]. The antifungal activity of EOs is associated with the presence of these compounds. EOs have mechanisms of action that allow them to interact with microbial membranes, causing cell lysis, inhibiting proton motor force, electron flow, transport active and protein synthesis [19]. Currently, more than 300 EOs are commercially available, including thyme, anise, citronella, clove, cinnamon, basil, thyme, lemon balm, tea, rosemary, and palmarosa [20]. Özcan [21] reported that the antagonist properties of some PEOs such as sage, bay, dill, cumin, fennel and thyme were effective in the control against *B. cinerea*. Muhammadi et al. [22] found that fennel, anise, peppermint and cinnamon oils increased the shelf life of fruits and inhibited the development of *B. cinerea* compared to the control. Antifungal effects of PEOs to control of *B. cinerea in vitro* were studied in apple [23], mango [24], citrus [25-26], sweet cherry and plum [27], kiwi [28-29], eggplant [30], strawberry [31-32], pepper [33], cucumber [34], tomato [35], table grapes [36]. However, a few study was found on the antifungal effect of PEOs against *B. cinerea* causing gray mold of eggplant in the literature reviews. The objective of this study was to determined the antifungal effect of PEOs of rosemary, black cumin, cumin, sandalwood and ginger on mycelial growth of *B. cinerea* under *in vitro* conditions.

2. Materials and Methods

2.1. Pathogenic fungal isolate of *B. cinerea*

Highly pathogenic fungal isolate of *B. cinerea* (ET 33) causing grey mold was isolated in pure culture from eggplant, showing grey mould (blight) symptoms and identified according to morphological, cultural and molecular biology in previous work [37]. Fungal isolate was aseptically subcultured and purified by serial transfers onto Petri plates containing 20 mL of potato dextrose agar (PDA-Difco). Plates were incubated in the dark at 25±1°C for 7 days and culture was stored at 4°C for in the refrigerator. Pathogen culture was prepared for experiment and was left to grow in the dark at 25±1°C for 7 days in an incubation chamber, before being used *in vitro* experiment.

2.2. Plant essential oils

In the study, five PEOs including rosemary, black cumin, cumin, sandalwood and ginger were selected based on their antifungal activity in the previous studies (Table 1). PEOs were produced by Arpaş Arifoğlu Co. (İstanbul, Türkiye) by steam distillation method. PEOs were stored at 4°C in a sealed vial until used.

Table 1: Basic data of PEOs used in the present study

Common name	Scientific name	Plant family	Brand name
Rosemary	*Rosmarinus officinalis* L	Lamiaceae	Rosemary Oil
Black Cumin	*Nigella sativa* L.	Ranunculaceae	Black Cumin Oil
Cumin	*Cuminum cyminum* L.	Apiaceae	Cumin Oil
Sandalwood	*Arbutus andrachne* L.	Ericaeeae	Sandalwood Oil
Ginger	*Zingiber officinale*	Zingiberaceae	Ginger Oil

2.3. Analysis of PEOs by gas chromatography-mass spectrometry (GC-MS)

Gas chromatography and mass spectrometry (GC-MS) (Shimadzu 2010 SE, Kyoto Japan) analysis were done at at Süleyman Demirel University Innovative Technologies Application and Research Center. Components of PEOs were identified by comparing linear retention indices (LRI) as well as comparing mass spectra with the Wiley library (Wiley, New York, NY, USA) and the NIST mass spectral database (Gaithersburg, MD, USA) [38].

2.4. Antifungal activity of PEOs under *in vitro* conditions

The antifungal test of PEOs carried out for assessing its contact phase effect towards mycelial growth of *B. cinerea* as described previously [39]. For determination of contact phase effect, various concentrations (0, 200, 400, 600, 800 and 1000 μL L^{-1}) of PEOs were prepared by dissolving them in Tween 20 (1:1) and added to flasks containing molten PDA. Nearly 20 mL of enriched media was poured into each plastic Petri plate (100 mm). A fungal disc (5 mm in diameter) were cut from the edge of one-week-old culture of *B. cinerea* grown on PDA, and placed in the center of each Petri plate [40]. The plates without the PEOs were used as control teratment. All Petri plates were sealed using parafim, incubated in the dark at 25±1ºC for 10 days. The experiment was carried out in a randomized plot design with three replications. The diameter of developed colonies was measured when fungal mycelium covered one plate in control treatment to calculate the inhibition effect. The inhibitory percentage at each concentration was calculated by the following formula (Equation 1):

$$IP = [(dc-dt) / dc] \times 100 \quad (1)$$

Where IP was inhibitory percentage, dc was the mycelium diameter in a control Petri plate, and dt was the mycelium diameter in the PEOs-treated Petri plate [27].

2.5. Statistical analysis

Data were analyzed by using JMP IN packet statistic program (SAS Institute, Carry, NC, 13.0 PC version). Analysis of variance (ANOVA) was applied to the values regarding the obtained *in vitro* test results, and the differences between the averages were compared with the LSMeans Differences Student's test at the significance level of $P \leq 0.01$.

3. Results and Discussion

3.1. Chemical composition of PEOs

The results obtained by GC-MS analyses of the five PEOs used in this study are presented in Table 2. A total of 80 active substances were determined in five different PEOs. Eucalyptol (1,8-cineole) (24.02%) was the main compound in *R. officinalis* PEO, followed by α-pinene (20.06%) and isoborneol (15.84%). In *N. sativa* PEO, eucalyptol (1,8-cineole) and α-pinene were the most abundant, representing 48.28%, 14.78% of the total compositions. The compounds in the *C. cyminum* PEO was found to be rich in cuminaldehyde (31.44%), γ-terpipene (17.79%) and cymol (14.73%). The highest percentage of compounds in the *A. andrachne* PEO were cedrene (18.63%), eucalyptol (1.8-cineole) (15.10%) and ethyl phthalate (7.23%). *Z. officinale* PEO was found to contain mainly sesquithujene (17.72%), eucalyptol (16.27%) and limonene (12.72%).

Biological activities of EOs depend on the qualitative and quantitative of their chemical components, plant genotype, plant chemotype, geographical origin, season, environmental factors and agronomic conditions [42]. It has been reported that eucalyptol (1.8-cineole), the main component of *N. sativa* EO, completely inhibits the growth of various pathogenic bacteria and fungi [43]. Phenolic components and EO of cumin are used as natural antioxidants [44]. Studies have shown parallelism with our results and it has been reported that antifungal activity of the *C. cyminum* EO may be attributed to cumin aldehyde and α-terpinen-7-al [45]. Ayodele et al. [46]

reported that phenolic compounds such as Sesquithujene, Eucalyptol (1,8-cineole) and Limonene isolated from *Z. officinale* EO inhibit the growth of phytopathogenic fungi.

Table 2: Chemical composition of rosemary (*R. officinalis*), black cumin (*N. sativa*), cumin (*C. cyminum*), sandalwood (*A. andrachne*) and ginger (*Z. officinale*) PEOs

			R. officinalis[c]	*N. sativa*[c]	*C. cyminum*[c]	*A. andrachne*[c]	*Z. officinale*[c]
No	**Compound**[a]	**LRI**[b]	**% of the essential oil**				
1	Tricyclene	924	1.05	0.77	0.04	0.21	1.44
2	alpha- Thujene	927	0.23	2.40	0.32	0.27	0.16
3	alpha - Pinene	933	20.06	14.78	1.68	4.90	7.01
4	beta- Fenchene	942	0.10	-	-	-	0.12
5	Camphene	953	5.02	4.77	0.24	1.62	12.33
6	Sabinene	972	1.15	2.23	0.32	0.61	0.51
7	beta- Pinene	978	2.96	9.07	16.03	2.96	2.11
8	4-Methyl-1-hepten-5-one	986	-	-	-	-	0.54
9	beta- Myrcene	991	0.48	-	0.69	-	1.55
10	Octanal	1006	-	-	-	-	0.14
11	Phellandrene	1007	0.16	-	0.44	-	0.39
12	DELTA.3-Carene	1009	0.45	-	0.05	-	0.04
13	alpha- Terpinene	1018	0.72	-	0.18	-	0.13
14	Cymol	1025	4.68	8.41	14.73	1.85	1.17
15	Limonene	1030	8.66	3.10	1.19	1.36	12.72
16	Eucalyptol (1,8-Cineole)	1052	24.02	48.28	1.93	15.10	16.27
17	gamma-Terpinene	1058	2.10	3.36	17.79	1.41	0.70
18	trans-Sabinene hydrate	1088	-	0.52	0.03	0.26	0.20
19	alpha- Terpinolen	1096	0.35	-	0.13	-	0.28
20	Dimethylstyrene (alpha-para)	1104	-	-	0.52	-	0.65
21	Linalool	1114	1.94	-	-	-	-
22	Chrysanthenone	1133	-	-	-	0.34	-
23	Carveol	1152	-	-	0.10	-	-
24	Camphor	1157	2.01	2.32	0.09	0.93	0.87
25	Isoborneol	1165	15.84	-	-	-	-
26	4-Terpineol	1193	0.49	-	0.28	-	0.18
27	Dimethylbenzylcarbinyl acetate (DMBCA)	1200	-	-	0.35	-	0.62
28	alpha- Terpineol	1207	4.31	-	-	-	-
29	Perilla alcohol	1208	-	-	0.85	-	-
30	Dihydrocarvone	1210	-	-	0.11	-	-
31	alpha- Terpinyl acetate	1213	0.60	-	-	-	-
32	Z-Citral	1238	-	-	-	-	1.88
33	Cuminaldehyde	1247	-	-	31.44	-	-
34	Carvotanacetone	1260	-	-	0.33	-	-
35	E-Citral	1268	-	-	-	-	2.25
36	Phellandral	1277	-	-	0.34	-	-
37	Bornyl acetate	1287	2.00	-	-	-	-
38	2-Undecanone	1294	-	-	-	-	0.17
39	2-Caren-10-al	1298	-	-	6.84	-	-
40	1-Phenylpropane-1,3-diol	1302	-	-	0.89	-	-
41	Thymol	1307	-	-	0.10	-	-
42	Carvacrol	1317	-	-	0.05	-	-
43	R(+)-Limonen	1358	-	-	-	2.93	-
44	Citronellyl acetate	1363	-	-	-	-	0.37
45	alpha- Copaene	1375	-	-	-	-	0.25
46	Hydrocoumarin	1386	-	-	-	5.93	-
47	gamma- Cadinene	1388	-	-	0.09	-	-
48	Linalyl acetate	1392	-	-	-	-	0.93
49	beta- Elemene	1400	-	-	-	-	0.33

50	alpha- Zingiberene	1414	-	-	-	-	0.09
51	Caryophyllene	1428	0.63	-	0.12	-	-
52	Coumarin	1438	-	-	-	5.94	-
53	Germacrene B	1439	-	-	-	-	0.15
54	Farnesene ((E)-, beta)	1466	-	-	0.05	-	-
55	alpha- Cedrene	1483	-	-	1.32	-	-
56	Germacrene D	1490	-	-	-	-	0.45
57	Curcumene	1491	-	-	-	-	4.30
58	Alloaromadendrene	1503	-	-	-	-	0.52
59	Sesquithujene (7-epi)	1506	-	-	-	-	17.72
60	alpha- Farnesene	1517	-	-	-	-	1.40
61	beta- Bisabolene	1519	-	-	-	-	3.80
62	beta- Sesquiphellandrene	1534	-	-	-	-	3.80
63	Ethyl phthalate	1592	-	-	-	7.23	-
64	Carotol	1601	-	-	0.05	-	-
65	Cedryl methyl ether	1624	-	-	-	4.56	-
66	Widdrene	1717	-	-	-	2.30	-
67	alpha.-Hexylcinnamaldehyde	1754	-	-	-	1.35	-
68	Cedrene	1768	-	-	-	18.63	-
69	Cedryl acetate	1771	-	-	-	2.48	-
70	beta- Guaien	1777	-	-	-	0.85	-
71	Aromadendrene	1790	-	-	-	0.39	-
72	Hexamethyl-pyranoindane	1847	-	-	-	6.99	-
73	Benzene, 1-(1,1-dimethylethyl)-3,5-dimethyl-2,4,6-trinitro-	1852	-	-	-	1.11	-
74	Tetralin (6-Acetyl-, 1,1,2,4,4,7-hexamethyl-)	1855	-	-	-	4.00	-
75	Musk ketone	1961	-	-	-	3.48	-
76	Tetracosane	2400	-	-	0.07	-	-
77	Pentacosane	2500	-	-	0.08	-	-
78	Hexacosane	2600	-	-	0.07	-	-
79	Heptacosane	2700	-	-	0.06	-	-
80	Nonacosane	2900	-	-	0.02	-	-
			100	100	100	100	100

[a]Compounds listed in order their elution, [b]LRI: Linear retention index, [c]GC-MS analysis results are shared in the article of Ceylan et al. [41].

3.2. Antifungal effect of PEOs on the mycelial growth of *B. cinerea*

The *in vitro* activity of different concentrations (0. 200, 400 ,600, 800 and 1000 µL L^{-1}) of tested PEOs (rosemary, black cumin, cumin, sandalwood, and ginger) against *B. cinerea* is summarised in Table 3. In comparison to the control, it was discovered that the inhibitory effects of rosemary, black cumin, cumin, sandalwood, and ginger PEOs on mycelial growth of *B. cinerea* was statistically significant ($P \leq 0.01$). Depending on the dose increase, each PEO utilized in the study reduced *B. cinerea* mycelial growth at a different rate. The highest mycelial growth was observed on control plates, while the lowest mycelial growth was obtained with 1000 µL L^{-1} cumin PEO. High dose (1000 µL L^{-1}) treatment of rosemary PEO showed 49.9% effects on tested *B. cinerea.* In other doses of rosemary PEO, inhibition of mycelial growth of *B. cinerea* was between 13.9% and 26.8%. Application of 1000 µL L^{-1} in black cumin PEO had an effect of 50.5% against *B. cinerea*. In other doses of black cumin PEO, inhibition of mycelial growth of the pathogen was between 14.3% and 37.0%. The highest inhibitory effect in cumin PEO was determined against *B. cinerea* in the high dose treatment at 62.5%, while 800 µl L^{-1} dose treatment followed the next highest effect (45.2%). In other doses of cumin PEO, inhibition of mycelial growth of *B. cinerea* was between 14.9% and 35.7%. High dose treatment of sandalwood PEO showed 47.4% effects on tested *B. cinerea*. In other doses of sandalwood PEO, inhibition of mycelial growth of *B. cinerea* was between 8.1% and 33.1%. 1000 µL L^{-1} application of ginger PEO showed the highest antifungal effect against *B. cinerea* (49.6%). In four doses of ginger PEO, inhibition of mycelial growth of *B. cinerea* was between 13.7% and 37.7%. In the study, cumin PEO was determined as the most effective

essential oil inhibiting mycelial growth of *B. cinerea*, compared to rosemary, black cumin, sandalwood and ginger EOs. High dose application of cumin PEO showed a high inhibitory effect against *B. cinerea*, and the inhibitory effects of the other four PEOs were found to be close to each other, depending on the pathogen and dose (Table 3).

Table 3: Antifungal effect of different concentrations of five PEOs on inhibiting mycelial growth of *Botrytis cinerea*

PEOs	Concentration (µL L^{-1})	ET 33 isolate	
		Mycelial growth (mm)[1]	Growth inhibition (%)
R. officinalis	0 (Control)	42.0 a*	0.0
	200	36.1 b	13.9
	400	34.7 c	17.3
	600	31.3 d	25.4
	800	26.5 e	26.8
	1000	21.0 f	49.9
	$CV_{(0.01)}$	2.1	
N. sativa	0 (Control)	42.0 a	0.0
	200	35.9 b	14.3
	400	33.5 c	20.1
	600	29.3 d	30.2
	800	26.4 e	37.0
	1000	20.8 f	50.5
	$CV_{(0.01)}$	2.8	
C. cyminum	0 (Control)	42.0 a	0.0
	200	35.8 b	14.9
	400	31.7 c	24.6
	600	27.0 d	35.7
	800	23.0 e	45.2
	1000	15.8 f	62.5
	$CV_{(0.01)}$	1.4	
A. andrachne	0 (Control)	42.0 a	0.0
	200	38.6 b	8.1
	400	35.0 c	16.7
	600	30.7 d	27.0
	800	28.1 e	33.1
	1000	22.1 f	47.4
	$CV_{(0.01)}$	1.3	
Z. officinale	0 (Control)	42.0 a	0.0
	200	36.3 b	13.7
	400	34.2 c	18.7
	600	29.3 d	30.2
	800	26.2 e	37.7
	1000	21.2 f	49.6
	$CV_{(0.01)}$	1.5	

[1]Ten days after inoculation, the mean mycelial growth of *B. cinerea* was calculated. Based on three replicate plates, each observation. *Mean values followed by different letters within the column are significantly different according to LSD Test (P≤0.01). CV: Coefficient of variation.

Similar to our results, Daferera et al. [47] reported that rosemary, lavender, sage, and pennyroyal EOs presented less inhibitory activity against *B. cinerea*. Scientific studies have shown the antibacterial, antifungal, antiviral and immune system-improving effects of rosemary EO [48]. Soylu et al. [49] found that rosemary, origanum and lavender EOs caused the significant morphological degenerations of the fungal hyphae (*B. cinerea*).

Contrary to our results, Lopez-Reyes et al. [50] reported that a 1% volatile emulsion of rosemary EO has a high antifungal activity against *B. cinerea* and *Penicillum expansum*. Dogu and Zobar [51] found that rosemary, thyme and minth EOs were determined more effective to *B. cinerea*. In the study, black cumin PEO did not show high effects at the concentrations tested. Similar to our results, Popescu et al. [52] reported that black cumin, sea buckthorn and grape seed EOs did not show antifungal activity against *B. cinerea* and *Fusarium oxysporum* at any of the concentrations tested. In our study, high doses of cumin PEO strongly inhibited the mycelial development of *B. cinerea*. Smilar to our results, Hammer et al. [53] found that ß -pinene and terpinene components of *C. cyminium* oil showed antifungal effect against various fungi. Lee et al. [54] reported that *C. cyminum* EO inhibited *B. cinerea* with MIC (Minimum Inhibitory Concentration) value of 5 mL per petri plate. Yosefi and Hasanzadeh [55] reported that a spore suspension (1×10^5 spore mL^{-1}) of the cumin EO was more effective in controlling the fungus *B. cinerea* on strawberry fruits as compared with fennel EO. Similarly, the cumin PEO antifungal effects against *B. cinerea*, *Aspergillus niger* and *Penicillium expansum* fungal pathogens, revealed at concentrations of ≥ 750 µL L^{-1}, the mycelial growth of the tested fungi was inhibited entirely [56]. Sandalwood PEO inhibited the mycelial growth of *B. cinerea* at varying rates depending on the dose. In literature searches, no studies were found to determine the antifungal effect of sandalwood PEO on mycelial growth of *B. cinerea*. This is the first study to investigate the antifungal effect of *A. andrachne* against *B. cinerea*. In the study, the inhibition percentage decreased in parallel with the decreasing dose of ginger PEO. Kılınç and Dolar [57] found that 1/1 dose of ginger extract inhibited colony growth by 69.52%. Contrary to our results, ginger PEO was found high antifungal effects against *Fusarium verticillioides* and *Verticillium dahliae* [58-59].

4. Conclusions

In the present study, the antifungal activity of five PEOs (rosemary, black cumin, cumin, sandalwood, and ginger) was determined against eggplant gray mold disease agent *Botrytis cinerea* under *in vitro* conditions. The effect of PEOs against *B. cinerea* decreased due to decreasing concentrations. Cumin PEO presented the best inhibitory effect over the *B. cinerea* (ET 33 isolate) at high dose (1000 µL L^{-1}), the diameter of mycelial being at 15.8 mm. The inhibitory effect of cumin PEO can be attributed to the compound Cuminaldehyde. The lowest antifungal effect against pathogen was detected in the high dose treatment of sandalwood PEO, 47.4%. However, further studies are needed to explain the concentration and timing of application of selected PEOs and the commercial formulation for successful eggplant gray mould disease control in detailed field experiments.

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