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ORIGINAL PAPER

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THE POTENTIAL APPLICATION OF *Origanum dubium* Boiss. ESSENTIAL OIL AS A SEED PROTECTANT AGAINST BEAN AND TOMATO SEED-BORNE BACTERIAL PATHOGENS

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ABSTRACT

Origanum dubium is a valuable wild oregano species of the natural flora of Antalya, Turkey. In this study, we extracted essential oil (EO) by hydro-distillation of the aerial parts of selected *O. dubium* chemotype with highest EO content, and analyzed by gas chromatography/mass spectrometry. Carvacrol was the primary component (85.9%) among 24 different compounds in the EO. The volatile test showed minimum inhibitory effect of the EO against *Xanthomonas axonopodis* pv. *vesicatoria*, *Clavibacter michiganensis* subsp. *michiganensis*, *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans*, and *Pseudomonas syringae* pv. *tomato*, at 309, 303, 318, 254, and 901 µL/mL, respectively. A 1-hour treatment of bean and tomato seeds in the volatile phase of the EO effectively sterilized the seeds from bacterial pathogens without inhibiting their germination. To the best of our knowledge, this is the first study on the potential for the use of *O. dubium* EO as a seed protectant against bean and tomato seed-borne bacterial pathogens.

Key words: antibacterial, bean, essential oil, *Origanum dubium*, seed-borne bacteria, tomato

INTRODUCTION

Origanum dubium Boiss. is distributed in the natural flora of Turkey, Greece and Cyprus. Recently, the taxonomic uncertainties concerning section *Majorana* have been resolved [Lukas et al. 2010]. Karioti et al. [2006] investigated its antimicrobial and potential antioxidant activities. The antimicrobial efficacies of *O. dubium* EO are closely associated with its carvacrol content that is a natural monoterpene phenol. Anticancer, antioxidant, anti-insect and antimicrobial activities of carvacrol have been reported by Koparal and Zeytinoglu [2003], Tang et al. [2011], Nostro and Papalia [2012]. Various biological activities of carvacrol from *Origanum*, *Thymbra*, *Thymus*, *Satureja*, and *Coridothymus* species have been reported by Baser [2008], Suntres et al. [2015] and Alagawany et al. [2015].

The industry prefers *O. dubium* rather than other *Origanum* species because of *O. dubium* high EO yield. The essential oils of *Origanum* species have strong antimicrobial effect because of high content of thymol and carvacrol [Sivropoulou et al. 1996]. After all, significant variations have been reported in regard to the antimicrobial effects of essential oils. Many factors such as ecology, agronomic and postharvest applications may cause these different biological activities. All these variables influence the chemical constitu-

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ents of the plant essential oils [Salgueiro et al. 1977, McGimpsey and Douglas 1994]. The differences in the essential oil composition of *Origanum* are likely to influence their antimicrobial features [Vokou et al. 1993].

There has been a clear tendency for safer management strategies of plant diseases that are eco-friendly to the environment and are less threatening to human health. Researches on plant essential oil help to improve the new preventive measures for plant diseases that may partially substitute agrochemicals [Basim and Basim 2013].

Many spices and derivatives acting as antimicrobial compounds have been studied for their effects against economically important plant pathogenic bacteria [Basim et al. 2000, Ozcan and Erkmen 2001, Basim and Basim 2004]. Nevertheless, there is no report on the antibacterial effect of *O. dubium* essential oil on plant seed pathogens.

O. dubium is heavily collected from the natural flora of Antalya, Turkey for its essential and carvacrol content. However, there are two different chemotypes in different locations of Antalya province. These are carvacrol and linalool abundant chemotypes [Turgut et al. 2016].

The objective of this study was to investigate the potential application of the EO of selected *O. dubium* genotype as a seed-protectant agent against bean and tomato seed-borne bacterial pathogens.

MATERIAL AND METHODS

Bacterial cultures. Table 1 shows the plant pathogen bacteria used in this study. The bacterial isolates were obtained from the stock culture stored at −86°C in Luria-Berthani broth − LB (MERCK, Darmstadt, Germany) − with 30% glycerol at Plant Protection Department, Faculty of Agriculture at Akdeniz University. The bacterial pathogens were grown on nutrient agar – NA (MERCK, Darmstadt, Germany).

Isolation of essential oil. *Origanum dubium* genotype with the highest essential oil and carvacrol contents was identified within the wild flora of Antalya in Turkey (Gazipasa, 1372 m above sea level; 36°26'749"N, 32°28'266"E) by the clone selection method in our preliminary studies [Turgut et al. 2017]. The EO of the selected *O. dubium* was obtained by the Clevenger hydro-distillation method. The upper parts of selected *O. dubium* plants were dried at room temperature, and the dried plant materials (100 g) were hydro-distilled with double distilled water (2 L) for 3 hours. The EOs were stored at 4°C before use for both antibacterial and gas chromatography assays. The volumetric method (v/w) was used to measure the EO percentage of the samples.

GC-MS analysis. The EO samples diluted with hexane at 1 : 50 ratio were analyzed by GC-MS (Agilent 7890A-Agilent 5975C). The system was operated in the EI mode at 70 eV, and was programmed to rise from 60°C (10 min) to 250°C (10.5 min). Helium gas was performed at a flow rate of 0.8 mL/min. Samples consisting of 1 µL with 50 : 1 split rate were injected into the capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm). The program was finalized in 60 min. Chemical composition of *O. dubium* EO was confirmed using Adams, NIST and Wiley libraries. The Kovats method was used to determine the retention indices of the EO constituent.

Contact and volatile phase effects of the essential oil. Essential oil (EO) obtained from *O. dubium* was utilized for assessing its contact and volatile antibacterial effects on *Clavibacter michiganensis* subsp.

Table 1. Experimental plant pathogenic bacteria used

Bacteria		Source	Host
C. michiganensis subsp. *michiganensis*	*Cmm2*	Basım H.	tomato
X. axonopodis pv. *vesicatoria*	91-118	Basım H.	tomato and pepper
P. syringae pv. *tomato*	1933-TD49	Basım H.	tomato
X. axonopodis pv. *phaseoli*	RK-579	Kotan R.	bean
X.axonopodis pv. *phaseoli* var. *fuscans*	3660	NCPPB	bean

michiganensis (*Cmm*), *Pseudomonas syringae* pv. *tomato* (*Pst*), *Xanthomonas axonopodis* pv. *vesicatoria* (*Xav*), *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (*Xapf*). These seed pathogens of tomato and bean were grown in nutrient broth medium for 24 hours at 28°C, centrifuged, washed in sterile tap water and resuspended to obtain a concentration of 10^8 cfu/mL. A bacterial suspension of 2 mL nutrient broth medium was included in order to determine starting concentration of bacteria. The optical density (O.D.) of the suspensions was measured at 600 nm using a spectrophotometer (NanoDrop Thermo Fisher Scientific, Beverly, MA, USA) after incubation of suspensions at 27 ±1°C on stirring plate. In order to determine the contact effects of different concentrations of EO, sterile glass flasks were arranged containing from 100 to 2500 µL/mL. The required quantities of the oregano EO were first mixed with 10 µL of Tween 80 (0.05% of total suspension, 20 mL) and added to the nutrient broth medium to obtain different doses of the EO. The flasks containing 1×10^5 cfu/mL of the test bacterial cells suspended in the liquid medium (nutrient broth − NB; MERCK, Darmstadt, Germany) were incubated at 28°C. MIC (minimum inhibition concentration, i.e. O.D. of the concentration, remained the same as in the previous day) was confirmed by plating 100 µL of the suspensions from the incubation flasks onto NA plates and by the optical density method, respectively. Bacterial colonies on the NA plates were counted at 24 hours intervals for 3 days. Control flasks contained NB and 1×10^5 cfu/mL of the test bacteria. Glass Petri dishes of 100 mL capacity were used for the determination of volatile phase effects of EO. A 100 µL of the test bacteria solution containing 1×10^8 cfu/mL were plated onto Petri dishes containing NA and the plates were dried under a sterile hood. Different concentrations of the essential oil, from 10 to 250 µL corresponding to 100 to 2500 µL/mL air, were dropped onto the lids to prevent from releasing of EO from Petri dish. Sealed Petri dishes with their lids at the bottom position were incubated at 28°C for 3 days. Then, the seals were removed to release volatile EO. Petri dishes were kept for another 3 days to determine bactericidal effect of the EO by counting bacterial colonies that appeared on the nutrient agar. The MIC was confirmed according to the equation of regression analysis [Diamond et al. 1941].

Seed sample preparation. Tomato (*Solanum lycopersicum* L.) cultivar Talya F1 seeds (1000 grains) and bean (*Phaseolus vulgaris*) cultivar Asya seeds (100 grains) were treated with 70% ethanol for 1 min and then in 1% sodium hypochlorite for 3 min for surface-disinfection. All treated seeds were rinsed three times with sterile distilled water. The seeds on sterile Whatman paper were kept in a laminar flow chamber for overnight. The air-dried seeds were kept at 4°C until used.

Seed inoculation. The tomato disinfected seeds were inoculated with *Cmm*, *Pst*, *Xav* isolates, and the bean seeds were inoculated with *Psp*, *Xap*, and *Xapf* isolates. The bacterial suspensions using sterile distilled water were prepared from bacterial isolates on NA, homogenized and adjusted to 10^8 cfu/mL (OD_{600} = 0.08−0.12). The tomato and bean seeds were inoculated using vacuum-infiltration for 30 min with the bacterial suspension (10 mL), and then all seeds were dried in a sterile laminar flow chamber, and then dried seeds were stored at 4°C.

Antibacterial activity of the essential oil on seed treatment. Tomato and bean seeds were left to dry after being treated with bacterial solutions at a concentration of 10^8 cfu/mL. MIC concentrations calculated in the volatile effect test for each pathogen were then added to the lid sections, mixed for 10 min spans and incubated for 1 hour. The lids were changed after 1 hour and allowed to continue incubation for 48 hours at 27°C. Control treatment contained only water. Bacterial isolation from the experimental seed was performed and the seed was checked for contamination with the inoculated bacterial pathogen.

Test of seed germination effect. Seeds treated with MIC concentrations determined in the trial of 1 hour-volatile effect of the *O. dubium* EO were subjected to the germination test. The effect of treatments was determined by evaluating the incidence of seed-borne pathogenic bacteria and the percentage of germination of treated tomato and bean seeds. The seed germination test was performed following ISTA [1996]. Three hundred tomato and bean seeds were tested in three replications of 100 seeds each, using the between-paper method. Tomato and bean seeds were incubated at 28°C and RH > 85%. The percentage of germinated seeds was determined after a ten-day incubation period.

Statistical analysis. SSPSS v17 (SPSS Inc., USA) was used to analyze the results. Statistical analyses were considered significant at $p < 0.01$. Analysis of variance ANOVA and Tukey's HSD analyses were used to determine significance of differences among extract-concentration effects.

RESULTS

Chemical composition of the essential oil. Chemical constituents of samples with retention indices are shown in Table 2. In total, 24 kinds of constituents were identified, representing 99.44% of the EO, by GC–MSD analysis. Quantitatively, carvacrol was the major constituent, and the constituents including p-cymene, α-thujene, γ-terpinene, trans-sabinene hydrate, and myrcene came after. The EO has also high volume of the active monoterpene phenols and its monoterpene hydrocarbon precursors. All main components are typical for *Origanum* species, e.g. *O. dubium* Boiss., *O. onites* L., and *O. syriacum* L. [ISTA 1996]. According to the results, essential oil yield amounted to 8.5% (Tab. 2). The main constituent was carvacrol with an average proportion of 85.9% (Tab. 2).

Table 2. Chemical composition of *Origanum dubium* EO

Peak	RI	Compounds	Genotype B (%)
		essential oil yield (%)	8.5
1	1019	methyl-2-methylbutyrate	0.1
2	1030	α-pinene	0.5
3	1033	α-thujene	1.1
4	1080	camphene	0.1
5	1123	β-pinene	0.1
6	1168	myrcene	1.1
7	1179	α-phellandrene	0.1
8	1190	α-terpinene	0.6
9	1213	limonene	0.1
10	1223	1.8-cineole	0.3
11	1257	γ-terpinene	1.7
12	1283	p-cymene	3.9
13	1447	1-octen-3-ol	0.2
14	1470	trans-sabinene hydrate	1.1
15	1549	linalool	0.2
16	1560	cis-sabinene hydrate	0.3
17	1616	terpinen-4-ol	0.6
18	1625	β-caryophyllene	0.1
19	1712	α-terpineol	0.2
20	1717	borneol	0.3
21	1749	carvone	0.3
22	2143	spathulenol	0.1
23	2187	thymol	0.7
24	2220	carvacrol	85.9
		total identified (%)	99.7

RI – retention index

The contact and volatile antibacterial effect of *O. dubium* EO. The contact antibacterial activity of the *O. dubium* EO was determined from the spectrophotometry absorbance values (Tab. 3). The volatile antibacterial activity of *O. dubium* EO was more effective than the contact antibacterial activities. The population size of the inoculated tomato and bean bacterial pathogens in the volatile antibacterial test were significantly reduced compared to the control, which was not treated with the EO (Tab. 4).

In NB media, EO doses of 1635 µL/mL or greater significantly decreased *Xav* population. EO doses of 2268 µL/mL or greater, significantly decreased *Cmm* population. EO doses of 1760 µL/mL or greater, significantly decreased *Xap* population, and EO doses of 978 µL/mL or greater, significantly decreased *Xapf* population. On the other hand, *O. dubium* EO was not effective in reducing the population size of *Pst* in NB medium (Tab. 5).

The results indicate that *O. dubium* EO showed highly effective antibacterial activity against all tested bacterial pathogens in NB medium (MIC values: 978–2268 µL/mL), whereas it was inactive against *Pst*. The MICs in volatile antibacterial tests for tomato and bean seed pathogens were lower than those in the contact antibacterial tests, overall ranging from 254 to 901 µL/mL, depending on the specific bacterial pathogen (Tab. 5). The MICs in volatile test showed strong bactericidal effect against all tested bacterial pathogens. This antibacterial effect of the EO can largely be attributed to high carvacrol content in *O. dubium* EO (85.88%) and associated hydrocarbons (13.60%).

The antibacterial and seed germination effects of *O. dubium* EO on seed treatment. The MICs of *O. dubium* EO determined for each bacterial patho-

Table 3. *In vitro* contact antibacterial effect of *Origanum dubium* EO against *C. m.* subsp. *michiganensis*, *P. s.* pv. *tomato**, *X. a.* pv. *vesicatoria*, *X. a.* pv. *phaseoli* and *X. a.* pv. *phaseoli* var. *fuscans*

C. m. subsp. *michiganensis*				*X. a.* pv. *vesicatoria*				*X. a.* pv. *phaseoli*				*X. a.* pv. *phaseoli* var. *fuscans*			
Doses (µL/mL)	A	IR	CFU	Doses (µL/mL)	A	IR	CFU	Doses (µL/mL)	A	IR	CFU	Doses (µL/mL)	A	IR	CFU
Control	1.280	00.00	1.86×10^7	Control	2.117	00.00	8.2×10^6	Control	2.203	00.00	2.48×10^8	Control	1.471	00.00	1.47×10^7
100	0.931	27.26	NC	100	1.668	21.21	NC	1000	0.237	89.24	NC	250	0.685	53.44	NC
200	0.892	30.39	NC	200	1.400	33.87	NC	1200	0.058	97.37	NC	500	0.121	91.74	NC
500	0.883	31.01	NC	500	0.764	63.91	NC	1400	0.024	98.91	2.69×10^2	750	0.041	97.21	19
1000	0.705	44.92	NC	1000	0.281	86.73	NC	1600	0.013	99.41	13	1000	0.00	100.00	0
1500	0.186	85.46	NC	1500	0.070	96.69	1.48×10^2	1800	0.00	100.00	0	1250	0.00	100.00	0
2000	0.027	97.89	22	2000	0.000	100.00	0	2000	0.00	100.00	0	1500	0.00	100.00	0
2500	0.000	100.00	0	*Tss*	0.000	100.00	0	*Tss*	0.000	100.00	0	*Tss*	0.000	100.00	0
Tss	0.000	100.00	0	Str	0.000	100.00	0	Str	0.000	100.00	0	Str	0.000	100.00	0
Str	0.000	100.00	0												

* No *in vitro* contact antibacterial effect of *Origanum dubium* EO doses was detected on *Pseudomonas syringae* pv. *tomato*
A − absorbance (600 nm), cfu − colony forming unit (cfu/mL), IR − inhibition rate (%), NC − noncountable (cfu/mL)
Tss − *Thymbra spicata* var. *spicata* (100 µL/mL), Str − streptomycin (100 µg/mL)

Table 4. *In vitro* volatile antibacterial effect of *Origanum dubium* EO against *C. m.* subsp. *michiganensis*, *P. s.* pv. *tomato*, *X. a.* pv. *vesicatoria*, *X. a.* pv. *phaseoli* and *X. a.* pv. *phaseoli* var. *fuscans*

Doses (µL/mL)	*C. m.* subsp. *michiganensis*		*P. s.* pv. *tomato*		*X. a.* pv. *phaseoli*		*X. a.* pv. *phaseoli* var. *fuscans*		*X. a.* pv. *vesicatoria*	
	cfu	IR	cfu	IR	cfu	IR	cfu	IR	cfu	IR
Control	7.07×10^2	00.00	4.37×10^2	00.00	1.458×10^3	00.00	1.214×10^3	00.00	9.66×10^2	00.00
10	4.78×10^2	32.39	3.75×10^2	14.19	9.94×10^2	31.83	7.28×10^2	40.03	6.41×10^2	33.64
20	2.42×10^2	65.80	3.40×10^2	22.20	5.41×10^2	62.90	2.59×10^2	78.7	3.40×10^2	64.80
30	5	99.29	2.98×10^2	31.81	8.7×10^1	94.03	0	100.00	3.4×10^1	96.48
40	0	100.00	2.52×10^2	42.33	0	100.00	0	100.00	0	100.00
50	0	100.00	2.06×10^2	52.86	0	100.00	0	100.00	0	100.00
100	0	100.00	0	100.00	0	100.00	0	100.00	0	100.00
Tss	0	100.00	0	100.00	0	100.00	0	100.00	0	100.00

cfu − colony forming unit (cfu/mL), IR − inhibition rate (%), *Tss* − *Thymbra spicata* var. *spicata* (100 µL/mL)

Table 5. *In vitro* contact and volatile antibacterial effect of *Origanum dubium* EO against *C. m.* subsp. *michiganensis*, *P. s.* pv. *tomato*, *X. a.* pv. *vesicatoria*, *X. a.* pv. *phaseoli* and *X. a.* pv. *phaseoli* var. *fuscans*

Bacteria	The contact antibacterial effect			The volatile antibacterial effect		
	MIC	regression analysis	R^2	MIC	regression analysis	R^2
C. m. subsp. *michiganensis*	2268^d	Y = 20.601 + 0.035•X	0.932	303^b	Y = 86.36 + 0.045•X	0.943
P. s. pv. *tomato*	-	-	-	901^e	Y = 49.145 + 0.056•X	0.917
X. a. pv. *phaseoli*	1760^c	Y = 84.162 + 0.009•X	0.912	318^d	Y = 84,735 + 0.048•X	0.954
X. a. pv. *phaseoli* var. *fuscans*	978^a	Y = 27.650 + 0.074•X	0.960	254^a	Y = 60.390 + 0.155•X	0.932
X. a. pv. *vesicatoria*	1635^b	Y = 21.490 + 0.048•X	0.870	309^c	Y = 57.66 + 0.137•X	0.856

MIC − minimum inhibitory concentrations (µL/mL) determined based on regression analysis; $p \leq 0.01$ according to Tukey's -HSD test
R^2 – multiple correlation coefficient

Table 6. *In vivo* volatile effect of *Origanum dubium* EO treatment on tomato and bean seeds and antibacterial effect against *C. m.* subsp. *michiganensis, P. s.* pv. *tomato, X. a.* pv. *vesicatoria, X. a.* pv. *phaseoli* and *X. a.* pv. *phaseoli* var. *fuscans*

Bacteria	Seed treatment concentrations (µL/mL)*	Seed germination (%)			Antibacterial effect	
		control	1-hour treatment	24-hour treatment	control (IR)	treatment (IR)
C. m. subsp. *michiganensis*	303[b]	100	100	0	0	100
P. s. pv. *tomato*	901[e]	100	100	0	0	100
X. a. pv. *phaseoli*	318[d]	100	100	0	0	100
X. a. pv. *phaseoli* var. *fuscans*	254[a]	100	100	0	0	100
X. a. pv. *vesicatoria*	309[c]	100	100	0	0	100

* MIC − minimum inhibitory concentrations (µL/mL) determined based on regression analysis, $p \leq 0.01$ according to Tukey-HSD test
IR − inhibition rate (%)

gen in the volatile antibacterial tests showed significant antibacterial effects on eliminating the tested seed-borne bacterial pathogens from bean and tomato seeds. The 1-h treatment of bean and tomato seeds in the volatile phase of the EO in a sterilized and sealed plastic container was sufficient to completely purify the seeds from bacterial pathogens without affecting the seed germination (Tab. 6). Bean and tomato seeds showed 100% germination, similar to the control seeds, that were treated with sterile ddH_2O water only, whereas the 24 hours EO treatment completely purified the seeds from bacterial pathogens, but drastically reduced the seed germination (Tab. 6).

DISCUSSION

In the present study, the calculated MIC for the *O. dubium* EO volatile phase was 100% effective against economically important bean and tomato seed-borne bacterial pathogens tested in these experiments, whereas the EO did not show any inhibitory effect on seed germination. Results confirmed strong antibacterial effect of the EO volatile phase against *Cmm*, *Xav*, *Xap*, and *Xapf* and *Pst*.

Investigating the antimicrobial effect, the mode of action, and potential use of EOs has gained importance, in parallel to the progress in traditional approaches to plant protection against microbes. For this reason, research upon antimicrobial efficacy of essential oil towards different plant microbes has experienced worldwide expansion over the last decades [Andrade et al. 2014]. Similarly, antimicrobial effect of EO of other *Origanum* species [Sivropoulou et al. 1996, Aligiannis et al. 2001] has been previously reported. Antimicrobial plant essential oils can have a major role in combating the plant diseases [Basim et al. 2000, Basim and Basim 2004].

The *O. dubium* EO yield was higher than that of other studies, amounting to 7.6% [Sarer et al. 1982] and 6.5–7.7% [Ahmad et al. 2011]. Carvacrol content of selected genotype was higher than that in previous studies on *O. majorana* from Turkey, 78.3 to 79.5% [Baser et al. 1993] and *O. dubium* from Cyprus, 69.5 to 71.3% [Karioti et al. 2006]. Essential oils abundant in carvacrol-like phenolic compounds have high antimicrobial effect [Sivropoulou et al. 1996, Aligiannis et al. 2001]. Although carvacrol has significant antioxidant and antimicrobial effects, it should be noted that all chemical components in an essential oil contribute to the antimicrobial effect synergistically [Stefan et al. 2013].

Previous studies have shown that Gram-positive bacteria were found to be more sensitive to the EO than Gram-negative bacteria [Betoni et al. 2006, Andrade et al. 2014]. In agreement with this observation, in our volatile effect test, *Cmm*, a Gram-positive bacterium, was found to be more sensitive than three Gram-negative bacteria including *Pst*, *Xap* and *Xav*.

Nguefack et al. [2013] demonstrated the efficacy of the EO against seed pathogens encountered in rice seeds. Seed health tests in this present study have shown that *O. dubium* EO was highly effective against tomato and bean seed-borne bacterial pathogens. The presence of a pathogen-contaminated seed in 10,000

seeds has the potential to cause an epidemic. For this reason, seed cleansing is of great importance in the combat against the plant bacterial diseases tested in these experiments.

Plant diseases cause economically significant yield losses [Hirano and Upper 1983]. Therefore, many kinds of agrochemicals have been used to prevent plant diseases. The increased residue problems on foods due to the use of agrochemicals have opened the way to search for alternatives of biological origin for plant disease control. Bactericides of plant origin can be an important alternative for plant disease management [Bolkan and Reinert 1994].

CONCLUSIONS

The results suggest the potential use of the *O. dubium* EO or its component, carvacrol, as tomato and bean seed protectants, due to their strong antibacterial activity. Since the use of pathogen-free seed in agricultural production is the most important step in combating the seed-borne bacterial pathogens, the effectiveness of the 1-hour natural seed treatment with *O. dubium* EO demonstrated in present study can be applied to eliminate bacterial pathogens from bean and tomato seeds without any negative effect on seed germination. To the best of our knowledge, this is the first study on the potential for use of *O. dubium* EO as a seed protectant against bean and tomato seed-borne bacterial pathogens.

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Conflict of interest. We have declared that there is no conflict of interests in the study.

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