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Effect of Essential Oil of *Origanum rotundifolium* on Some Plant Pathogenic Bacteria, Seed Germination and Plant Growth of Tomato

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Abstract. The aim of this study is to determine effect of *Origanum rotundifolium*'s essential oil on some plant pathogenic bacterias, seed germination and plant growth of tomato. *Xanthomonas axanopodis* pv. *vesicatoria* strain (Xcv-761) and *Clavibacter michiganensis* ssp. *michiganensis* strain (Cmm) inoculated to tomato seed. The seeds were tested for germination in vitro and disease severity and some plant growth parameters in vivo. In vitro assay, maximum seed germination was observed at 62,5 µl/ml essential oil treatment in seeds inoculated with Xcv-761 and at 62,5 µl/ml essential oil and streptomycin treatment in seeds inoculated with Cmm. The least infected cotiledon number was observed at 500 µg/ml streptomycin treatment in seeds inoculated with Cmm. In vivo assay, maximum seed germination was observed at 250 µl/ml essential oil teratment in tomato inoculated with Cmm. Lowest disease severity, is seen in the CMM infected seeds with 250 µl/ml essential oil application these results were statistically significant when compared with pathogen infected seeds. Similarly, in application conducted with XCV-761 infected seed, the lowest disease severity was observed for seeds as a result of 250 µl/ml essential oil application. Also according to the results obtained from essential oil application of CMM infected seeds conducted with 62,5 µl/ml dose; while disease severity was found statistically insignificant compared to 250 µl/ml to essential oil application, ıt was found statistically significant compared to pathogen infected seeds. The results showed that essential oil of *O. rotundifolium* has a potential for some suppressed plant disease when it is used in appropriate dose.

INTRODUCTION

Avoiding or mitigating crop losses due to plant diseases caused by pathogenic bacteria, fungi and viruses is one of the most important issues in plant production. Nowadays, rapid and effective control of fungal plant disease and microbial contamination in the crops is generally achieved using synthetic pesticides and, sometimes, antibiotics. The control of plant bacterial diseases remains difficult due to limited availability of commercial bactericides and prohibition to use antibiotics in many countries. Thus, chemical control of bacterial diseases is largely dependent on the use of copper compounds. However, such control methods prevent bacterial multiplication but are not adequate for seed-borne inocula. Furthermore, chemicals of this type evoke undesirable effects on the environment and leave residues toxic to mammalians in the products [1, 2]. The risk of

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developing resistance in microorganisms and the high cost-benefit ratio are other disadvantages of synthetic pesticides [3, 4].

The genus *Origanum* (oregano), family Lamiaceae, is represented in Turkey by 22 species, 21 being endemic to the country. *Origanum* species are aromatic and are used as condiment or herbal tea [5, 6]. Although there are numerous reports on the chemical composition and antibacterial activity of some *Origanum* species against various bacteria of food, clinical and plant origin [7, 8, 9, 10, 11, 12, 13, 14]. Objectives this study to determine effect of *Origanum rotundifolium*'s essential oil on some plant pathogenic bacterias, seed germination and plant growth. Antibacterial effect *Origanum rotundifolium*'s essential oil on used bacterial strains was tested in our previous study [18].

MATERIAL AND METHODS

Bacterial Strains and Plant Materials

Three bacterial strain was used from culture collection of Assoc. Prof. Dr. Recep Kotan (Ataturk University, Agricultural Faculty, Turkey). Whole strains were tested formerly and they were highly virulance strains. The aerial parts of *O.rotundifolium* were collected in Erzurum (Turkey) during flowering between July- September 2008, and were air dried in the shade. Specimens of all three species were deposited in the herbarium of Ataturk University, Erzurum.

Isolation of The Essential Oil and Seed Surface Disinfection

Dried plant samples (500 g) were subjected to hydrodistillation using a Clevenger- type apparatus for 4 h. Oils were extracted with $CHCl_3$ and then dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20°C in a sealed vial until use. Essential oil yields from *O. rotundifolium* 0.60% (w/w) [15].

The seeds were surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the seed surface. Seed disinfection was performed by dipping the seeds for 3 min in 3 % sodium hypochlorite and washing four times in sterilized and distilled water (sd. H_2O). Seeds were left to dry on sterile. Whatman filters paper sheets incubated overnight in the laminar flow hood for using further studies.

Seed Inoculation of Pathogens and Essential Oil

Essential oil solutions, prepared different concentration with 10% DMSO (1/5, 1/10, 1/25, 1/50, 1/100, 1/125, 1/250, 1/500 ve 1/1000 v/v), was added sterile tubes. Bacterial cultures were grown in nutrient broth (NB) for 24 hours. Absorbance of bacterial suspension was measured spechtrophotometrically at 600 nm and appropriately diluted $1x10^8$ CFU / ml. 100 µl from this suspension were added to tubes, included essential oil and pathogens. Then 40 seeds was added to tubes and incubated on hematology shaker for 24 hours. After incubation, the seeds were removed and air-dried on sterile. Whatman filters paper sheets incubated overnight in the laminar flow hood.

Determining Effect of Essential Oil Treatment on Disease Severity, Seed Germination and Plant Growth

This experiment was performed in two ways. First, after traitment, 10 seeds were transferred to petri dishes with sterile whatman filter paper. Filter paper full with sdH_2O. Then petri dishes were coated with parafilm and incubated at room temperature. Germination was recorded daily and at the end of 20th day, cotiledon leaf was checked and infectous plants were recorded.

Secondly, after the traitment above, 10 seeds were transferred to pots and grown in chamber at 24–28 °C and 90 % humidity. Disease severity and germating seed number were recorded. 1-5 scale was used for disease severity (1: no symptom; 2: symptoms in 25% of the leaves; 3: symptom in 50 % of the leaves; 4: symptoms in 75 % of the leaves; 5: symptoms in 100 % of the leaves). Additionally, plant height, stem and rot weight were recorded. In order to determine whether there is a statistically significant difference among the results obtained from antibacterial effect of tested plant essential oils, variance analyses were carried out using SPSS 15.0 program. Values of $p<0.05$ were considered as significantly different. All applications were repeated 3 times.

RESULTS

In terms of seed germination in petri dishes, maximum seed germination was observed from 62,5 µl/ml essential oil treatment for *Xanthomonas axanopodis* pv. *vesicatoria* in tomato (table 1) and from 62,5 µl/ml essential oil and streptomycin treatment for *Clavibacter michiganensis ssp. michiganensis* in tomato (table 3) at the end of the 20th day.

Considering infected cotiledon ratio, least infected cotiledon was observed from 500 µg/ml streptomycin treatment for *Xanthomonas axanopodis* pv. *vesicatoria* in tomato. This was statistically important from steril seed and steril seed with pathogen treatments (table 1). The same results were observed for *Clavibacter michiganensis ssp. michiganensis* in tomato (table 3). This was also statistically important from steril seed and steril seed with pathogen treatments (table 3).

For *Xanthomonas axanopodis* pv. *vesicatoria* in tomato, maximum seed germination was observed streptomycin treatment and this was statistically important from steril seed and steril seed with pathogen treatments (table 2). For *Clavibacter michiganensis ssp. michiganensis* in tomato, maximum seed germination was observed at 250 µl/ml essential oil teratment and this was statistically important from steril seed and wasn't important from steril seed with pathogen (table 4). For *Xanthomonas axanopodis* pv. *vesicatoria*, least disease severity was observed in 250 µl/ml essential oil treatment in tomato and this was statistically important from steril seed and steril seed with pathogen (table 2). And least disease severity of *Clavibacter michiganensis ssp. michiganensis* was observed in 250 µl/ml essential oil treatment in tomato and this was statistically important from steril seed with pathogen and wasn't important steril seed treatments (table 4).

According to statistical analysis, for *Xanthomonas axanopodis* pv. *vesicatoria* in tomato, maximum values were observed from steril seed for plant height and in 125 µl/ml essential oil treatment for stem and rot weight. The increase of stem weight was important from steril seed treatment. Essential oil treatment generally increases germination ratio, rot and stem weight (table 2). And for *Clavibacter michiganensis ssp. michiganensis* in tomato, maximum values were observed from steril seed treatment for plant height, from streptomycin treatment for rot weight and from 62,5 µl/ml essential oil treatment for stem weight and the increase of stem weight was important from steril seed treatment. Similarly, essential oil treatment generally increases germination ratio and stem weight (table 4).

TABLE 1. Effect of different concentration treatment of *Origanum rotindufolium*'s essential oil on enfected tomato seed with *Xanthomonas axanopodis* pv. *Vesicatoria* Xcv- 761 in petri dishes.

Treatment	Germinated seed number and Germination ratio (%)				Infected cotiledon ratio (%)
	5. day	10. day	15. day	20. day	
250 µl/ml essential oil	5.67±0.42 a	5.67±0.42 a	5.67±0.42 a	56.7	100 b
125 µl/ml essential oil	7.00±0.36 ab	7.33±0.21 bc	7.33±0.21 bc	73.3	100 b
62.50 µl/ml esential oil	**8.00±0.36 b**	**8.00±0.36 c**	**8.00±0.36 c**	**80.0**	91.6 b
500 µg/ml Streptomycin	6.33±0.55 a	6.33±0.55 ab	6.67±0.42 a-c	66.7	**59.9 a**
Steril seed with pathogen	7.00±0.63 ab	7.00±0.63 a-c	7.00±0.63 a-c	70.0	100 b
Steril seed	6.33±0.55 a	6.33±0.55 ab	6.33±0.55 ab	63.3	94.7 b

500 µg/ml streptomycin, steril seed with pathogen and steril seed were used as control.

TABLE 2. Effect of different concentration treatment of *Origanum rotindufolium*'s essential oil on infected tomato seed with *Xanthomonas axanopodis* pv. *Vesicatoria* strain Xcv-761, plant height, rot and stem weight in pots.

Treatment	Germination ratio (%)	Disease severity	Plant height	Rot weight	Stem weight
250 µl/ml essential oil	36.7 ab	**1.63±0.16 b**	21.36±0.84 ab	11.16±0.27 a	2.63±0.18 ab
125 µl/ml essential oil	50.0 ab	2.17±0.30 b	22.18±3.11 ab	**12.30±0.60 a**	**3.26±0.37 b**
62.50 µl/ml essential oil	50.0 ab	2.67±0.21 c	17.93±1.13 a	12.24±1.64 a	3.25±0.40 b
500 µg/ml Streptomycin	**53.3 b**	4.00±0.00 d	20.61±0.27 ab	12.13±0.47 a	3.24±0.12 b
Steril seed with pathogen	40.0 ab	4.00±0.00 d	20.38±1.34 ab	10.75±0.09 a	2.64±0.13 ab
Steril seed	26.7 a	**1.00±0.00 a**	**25.53±3.02 b**	10.54±0.40 a	1.93±0.39 a

Standart error is (p=0.05) ; Disease severity to 1-5 scale**:** (1: no symptom; 2: symptoms in 25% of leaves; 3: symptom in 50% of leaves; 4: symptoms in 75% of leaves; 5: symptom in 100% of leaves).

TABLE 3. Effect of different concentration treatment of *Origanum rotindufolium*'s essential oil on enfected tomato seed with *Clavibacter michiganensis* ssp. *michiganensis* strain Cmm in petri dishes.

Treatment	Germinated seed number and Germination ratio (%)				Infected cotiledon ratio (%)
	5. day	10. day	15. day	20. day	
250 µl/ml essential oil	7.00±0.36 bc	7.33±0.21 bc	7.33±0.21 bc	73.3	100 b
125 µl/ml essential oil	5.33±0.91 a	6.00±0.63 a	6.00±0.63 a	60.0	94.5 b
62.50 µl/ml essential oil	**7.67±0.21 c**	**7.67±0.21 c**	**7.67±0.21 c**	**76.7**	86.9 b
500 µg/ml Streptomycin	**7.67±0.21 c**	**7.67±0.21 c**	**7.67±0.21 c**	**76.7**	**52.1 a**
Steril seed with pathogen	6.00±0.36 ab	6.33±0.42 ab	6.33±0.42 ab	63.3	100 b
Steril seed	6.33±0.55 a-c	6.67±0.55 a-c	6.67±0.55 a-c	66.7	89.9 b

500 µg/ml streptomycin, steril seed with pathogen and steril seed were used as control.

TABLE 4. Effect of different concentration treatment of *Origanum rotindufolium*'s essential oil on enfected tomato seed with *Clavibacter michiganensis* ssp. *michiganensis* strain Cmm, plant height, rot and stem weight in pots.

Treatment	Germination ratio (%)	Disease severity	Plant height	Rot weight	Stem weight
250 µl/ml essential oil	**53.3 b**	**1.33±0.21 ab**	18.77±0.88 a	9.35±0.28 a	2.17±0.02 ab
125 µl/ml essential oil	36.7 ab	2.00±0.25 c	21.10±0.49 ab	8.99±0.19 a	2.09±0.05 ab
62.50 µl/ml essential oil	46.7 b	1.83±0.16 bc	23.41±0.69 ab	10.14±0.22 a	**2.97±0.05 b**
500 µg/ml Streptomycin	40.0 ab	2.33±0.21 c	21.27±1.69 ab	**12.75±0.86 a**	2.56±0.26 ab
Steril seed with pathogen	46.7 b	3.00±0.00 d	20.50±0.41 a	12.25±0.77 a	2.75±0.47 ab
Steril seed	26.7 a	1.00±0.00 a	**25.53±3.02 b**	10.54±0.40 a	1.93±0.39 a

Standart error is (p=0.05) ; Disease severity to 1-5 scale**:** (1: no symptom; 2: symptoms in 25% of leaves; 3: symptom in 50% of leaves; 4: symptoms in 75% of leaves; 5: symptom in 100% of leaves).

DISCUSSION

In recent years, the use of synthetic pesticides in plant disease protection programs around the world has resulted in disturbances of the environment, pest resurgences, and pest resistance to pesticides and lethal effect to nontarget organisms in the agro-ecosystems in addition to direct toxicity to users [16, 17, 18]. Therefore, considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms. The use of plant essential oil and extracts as antimicrobial agents is one of the first choices after plant bacterial disease outbreaks. Besides, interest has been generated in the development of safer antibacterial agents to control plant pathogenic bacteria in agriculture which also include essential oils and extracts [18]. In our study, it is detected that the essential oil of *O. rotundifolium* is effective against the seed-borne *X. a*. pv. *vesicatoria* and *C. m.* ssp. *michiganensis* pathogens when used in appropriate doses. In general, the essential oil treatment is led to an increase in the rate of germination and body weight with respect to the sterile seed treatment. It is considered that there is a linear relationship between the changes in plant height, root and stem weight and essential oil concentration besides the relation of inequality in the number of germinated plants in pots to the nonhomogenous distribution of vegetation.

Essential oils showed also bactericidal effects against most of the bacteria tested. The MIC values of the oils varied with the strains tested, ranging from 31.25 to 500 ml ml^{-1} confirming literature reports on the antimicrobial activity of *O. vulgare* and *O. acutidens* essential oils against various pathogens [7, 8, 9, 11, 13, 14, 19]. Likewise, [19] documented that the essential oil of Turkish *O minutiflorum* possesses strong antimicrobial activity against *X. vesicatoria*. According to our results, it is observed that the essential oil treatment in doses of 6,25, 125 to 500 mL statistically reduces the severity of diseases caused by the *X. a.* pv. *vesicatoria* and *C. m*. ssp. *michiganensis* pathogens which is compatible with the results in literature. The mechanism of the action of the essential oil is probably related to the outer membrane disintegrating properties of thymol and carvacrol

[20].This property of *Origanum* essential oils is apparently related to their high phenolic contents, particularly carvacrol and thymol [15]. Our results also confirmed that the potent antibacterial effect of the essential oil of *O. rotundifolium* is probably related to its carvacrol and thymol constituents. In the literature, some investigations suggest that these compounds penetrate inside the cell, where they interfere with cellular metabolism [21, 22].

As a result, it is considered that the appropriate doses of essential oil of O. rotundifolium can be used on tomato seeds against *X. a.* pv. *vesicatoria* and *C. m.* ssp. *michiganensis* pathogens as a potential agent while not having any negative effect on the germination and growth of tomato seeds. This study matters to highlight the successful usage of an environment-friendly, natural, risk free for health of humans and other livings product against some seed-borne pathogens in substitution for the chemical pesticides that are intensely used and harmful for environment, natural balance and human health.

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