

Effect of Essential Oils of Five Medicinal Plants on Two Microbial Diseases of Potato and Tomato under Laboratory and Field Condition

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Abstract – This study was conducted to assay the biocontrol efficiency of essential oils on two destructive plant diseases of potato and tomato, *in vitro* and under greenhouse conditions. In laboratory tests antibacterial effects of the essential oils extracted from *Coriandrum sativum*, *Thymus vulgaris*, *Cuminum cyminum*, *Rosmarinus officinalis* and *Eucalyptus globulus* in different concentrations were tested against *Pectobacterium carotovorum* (causal agent of soft rot in potato) and *Ralstonia solanacearum* (causal agent of bacterial wilt in potato and tomato). Thyme oil exhibited the highest antibacterial activity in cultured media for both plant pathogenic bacteria, so it was selected to apply in greenhouse experiments to determine bacterial wilt and soft rot incidence on potato and tomato. Treatment by thyme essential oil caused significant reduction in soft rot and bacterial wilt incidence on potato by 41 and 44%, respectively. The same treatment on tomato caused 50% reduction of wilt compared with the control sample. The obtained results showed that thyme oil had antibacterial effects on the tested bacteria on plate and under greenhouse conditions. Generally, the greenhouse results confirmed those of *in vitro* assays by reduction in bacterial wilt and soft rot incidence. However it showed stronger effects on controlling bacterial wilt on tomato.

Keywords – Bacterial Wilt, Soft Rot, Thyme Oil, Antibacterial Effects.

I. INTRODUCTION

Pectobacterium carotovorum pv. *carotovorum* and *Ralstonia solanacearum* are plant pathogens with a diverse host range, including potato and tomato. Soft rot and bacterial wilt caused by *P. carotovorum* pv. *carotovorum* and *R. solanacearum* respectively are considered as dangerous diseases of potatoes. These diseases reduce the yield of plant production. These are among the major diseases transmitted by infected tubers and seeds of potato. Certain control measures have not been identified for these diseases [1] and the most effective way is to avoid planting the infected tubers, which is not considered as a method of definite prevention. It is very difficult to control soil-borne diseases and efficiency of current methods to manage them is not significant [2]. Although using resistant cultivars is considered as an important part of integrated management but the process of creating resistant cultivars are long-term, costly and difficult [2]. In special geographic areas, the expression of resistant cultivars is often limited due to the influence of environmental factors on reactions of plant pathogens [2]. Moreover, for controlling plant bacterial disease, only copper compost and antibiotics, which are forbidden in a

large number of countries and their usage should be in compliance with some regulations in several other ones, are available on the market. As a matter of fact, there are no bactericides to be used in agricultural practice. In addition, using synthetic antimicrobial drugs/chemicals has not been identified as an efficient way regarding undesirable side effects [3]-[4]. So it is necessary to find biological control methods which are safe and environment friendly [5]. In recent years, using a large number of antimicrobial essential oils and their constituents has been considered as a reliable method of biological control [6]-[7]. Pradhanang *et al.* tested the bactericidal influence of thymol as a fumigant in soil and observed the reduction of bacterial wilt intensity on planted tomatoes. Bendaoud [9] indicated the inhibitory activity of eucalyptus on *Agrobacterium tumefaciens*. The antibacterial effects of thyme and eucalyptus on *R. solanacearum* [10] and thymol on *Xanthomonas axonopodis* [11] were investigated. El-Zemity [12] showed the growth of *P. carotovorum* was inhibited by thyme oil. Although there are several researches confirmed the antibacterial effects of medicinal plants on pathogenic bacteria, but it has been stated that the yield and composition of essential oils for each species is affected by diverse factors such as physiological variations, environmental conditions and geographic variations genetic factors [13]. Therefore, antimicrobial characteristics of native Iranian medicinal plants have to be subjected for research studies. The present study aimed at investigating the antibacterial activity of essential oils (EOs) extracted from *Coriandrum sativum*, *Thymus vulgaris*, *Cuminum cyminum*, *Rosmarinus officinalis* and *Eucalyptus globulus* which are endemic Iranian plants against *P. carotovorum* and *R. solanacearum* in order to find the best oil and concentration that inhibit bacterial growth *in vitro*. Greenhouse experiments were further conducted to test the results obtained *in vitro* assays on inoculated-potato and tomato plants.

II. MATERIALS AND METHODS

A. Bacterial cultures

Bacterial strains of *Pectobacterium carotovorum* subsp. *carotovorum* and *Ralstonia solanacearum* (race 3, biovar 2) were provided by The Department of Plant Pathology, Isfahan University of Technology, Iran.

B. Preparation of essential oils

Leaves of *Thymus vulgaris*, *Rosmarinus officinalis* and *Eucalyptus globulus* were collected from Agricultural

Institute of Zabol University. Seeds of *Cuminum cyminum* and *Coriandrum sativum* were obtained from Research Institute of Food Science and technology, Mashad, Iran. The dried herb samples (100 g) were ground and subjected to hydro distillation for 3 hours using a Clevenger-type apparatus. The distillate oils were dried over anhydrous sodium sulfate (Na_2SO_4) and preserved in darkness and sealed vials at 4 °C until use.

C. Laboratory tests

Agar disc diffusion method was employed for antibacterial assays. 0.1 ml of bacterial standardized suspensions containing 10^8 CFU/ml were inoculated on culture plates (9 cm) containing solid media. The inoculum was uniformly spread on the surface of plates and allowed to dry. Sartorius No.388 sterile filter paper disc (6 mm diameter) was dropped with 10 µg/disc of each EO solutions with concentrations of 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 75 and 100% in absolute ethanol. Negative control was prepared using the same solvent (absolute ethanol) while antibiotics of streptomycin (10 µg/disc) and erythromycin (15 µg/disc) were used as positive controls. The diameter of inhibition zones was measured after incubation at 28 °C for 24 h.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was performed according to the modified procedure of Kivanc and Akgul (14). Bacterial strains were cultured overnight in nutrient broth at 28 °C. Then, 5 ml volume of 10^8 CFU/ml microbial suspensions were incubated in a series of tubes containing 50 µg of each oil concentrations. After overnight incubation, MIC was defined as the lowest concentration with no visible growth. The MBC was defined by sub-culturing the tubes with no growth on agar plates to determine whether the inhibition was reversible or permanent. MBC was the lowest concentration which killed bacteria.

D. Growth of plants

The greenhouse pots were filled with soil mixture prepared from sand, compost, and clay at the ratio of 1:1:1. The soil was sterilized at 121 °C for 20 min and filled in a sterilized pot. Pots of 20 cm diameter were filled

with two kilograms sterile soil into which two eyepieces of potato (cultivar fontane) were planted. To plant tomato, sterilized soil was filled into each 6 cm diameter pots planted with one seed of tomato (cultivar mobile Hungary). The plants were maintained in a greenhouse at temperatures of 28–30 °C and 60% relative humidity and seedlings were watered when necessary. The plants were fertilized with NPK (16–16–16) as soil drenched with the fertilizer at a concentration of 340 g/ml every seven days.

E. Oil treatment

After one month of planting, soil of each pot was drenched with 100 ml of oil solution at concentration of 1000 µg per liter soil. The stable oil solution was prepared by dissolving pure oil in 5% dimethyl sulfoxide (DMSO) + 1% surfactant Tween 80 in water.

F. Bacterial contamination

48 hours after treatment with EOs, potato and tomato pots were contaminated with pathogenic strains. Soil of each pot was drenched with the overnight bacteria suspension (10^8 cfu/ml) at the rate of 100 ml/pot. Potato plants were inoculated with strain *R. solanacearum* and *P. carotovorum* separately, and tomatoes were inoculated with strain *R. solanacearum* by the same method [15]. There were four groups treatments in greenhouse experiments including first group untreated contaminated-plants (positive control), second group treated contaminated-plants with EO, third group treated non-contaminated plants (to investigate the allopathic properties of thyme oil on foliage or root of potato and tomato plants) and finally fourth group untreated non-contaminated plants (negative control).

G. Disease assessment

The plants started to show wilting and rotting about 10 day after inoculation while within twenty days after treatment, every symptom of wilting on potato and tomato were observed and recorded as well as all signs of soft rot on potato plants. Plant shoot and root weight were measured to evaluate effect of oil on plant growth. Roots were washed thoroughly to remove soil before measurement.

Table 1. Antibacterial activity of the essential oils against *R. solanacearum*

Essential oils	Concentrations(%)											
	0	0.01	0.05	0.1	0.5	1	5	10	25	50	75	100
<i>C. sativum</i>	0m	0m	0m	0m	0m	0m	0m	0m	6.6l	7.1k	7.8j	9.1h
<i>C. cyminum</i>	0m	0m	0m	0m	0m	0m	0m	6.5l	7.1k	8j	9h	9.6g
<i>T. vulgaris</i>	0m	0m	0m	6.5l	7.8j	8.5i	10.if	11.8e	13.1d	22.8c	29.6b	34.8a
<i>R. officinalis</i>	0m	0m	0m	0m	0m	0m	0m	0m	6.5l	8j	8.8hi	11.8e
<i>E. globulus</i>	0m	0m	0m	0m	0m	0m	0m	0m	0m	0m	0m	6.5l

Diameter of inhibition zones includes diameter of disc (6 mm)

Concentration %0 refers to paper discs containing only ethanol (Negative control)

values followed by the same letter indicates no significant difference according to Duncan's multiple range test at $P < 0.05$

The disease index of bacterial wilt was recorded based on a scale of 0–4 as described by Kempe and Sequeira [16] and disease index of soft rot was recorded based on a

scale of 0-8 that 0 for the health plants without any signs of rotting and 8 for dead plants. Disease incidence and biocontrol efficiency were calculated as follows:

Disease incidence = [P (The number of diseased plants in this index _ Disease index)/ (Total number of plants investigated _ the highest disease index)] ×100.

Biocontrol efficacy = [(Disease incidence of control _ Disease incidence of treatment)/Disease incidence of control] ×100.

The fresh weight data was taken by weighing the above and underground part, and the cuts were then oven-dried at 60 °C for 72 h, and dry weight was recorded. Furthermore, biomass increase was calculated with the following formula:

Biomass increase = [(Average weights of plants treated with oil_ Average fresh weights of control plants)/Average weights of control plants] ×100.

H. Statistical analysis

All tests were replicated three times. Data were carried out by analysis of variance (ANOVA) and the differences in mean were calculated for significance at $P < 0.05$ using Duncan's multiple range test (MSTATC software).

III. RESULTS

A. In vitro assay

Table 2. Antibacterial activity of the essential oils against *P. carotovorum*

Essential oils	Concentrations(%)											
	0	0.01	0.05	0.1	0.5	1	5	10	25	50	75	100
<i>C. sativum</i>	0n	0n	0n	0n	0n	0n	8hi	9.1g	10f	10f	10.6e	11.17d
<i>C. cyminum</i>	0n	0n	0n	0n	0n	0n	0n	6.6m	7.1kl	7.1kl	7.5jk	7.5jk
<i>T. vulgaris</i>	0n	0n	0n	0n	7.1kl	7.6ij	9.5g	10.5e	14c	15.6b	16b	16.5a
<i>R. officinalis</i>	0n	0n	0n	0n	0n	0n	0n	0n	6.6m	7.1kl	7.1kl	8.1h
<i>E. globulus</i>	0n	0n	0n	0n	0n	0n	0n	0n	0n	0n	6.8lm	7.6ij

Diameter of inhibition zones includes diameter of disc (6 mm)

Concentration %0 refers to paper discs containing only ethanol (Negative control)

values followed by the same letter indicates no significant difference according to Duncan's multiple range test at $P < 0.05$

B. Greenhouse experiments

Table 3, 4, 5 and 6 summarize the result of assaying biologically control of thyme oil on bacterial wilt and soft rot diseases under greenhouse conditions. Generally, there was significant reduction in bacterial wilt and soft rot incidence while plant shoot and root weights were increased. Root and shoot weights of negative controls and treated non-contaminated plants were not affected significantly. In this experiment, over 80% of untreated contaminated plants (positive control) wilted and showed symptoms of soft rot. Even though, none of untreated non-contaminated potatoes and tomatoes (negative control) wilted or rotted. Moreover, there were not any abnormal symptoms on foliage and root of treated non-contaminated plants.

According to the table 3, disease incidence was 40.67% in treated inoculated-potatoes by *P. carotovorum* compare to the positive control (82.33%). Likewise, by applying thyme oil in potato soil, dry and fresh weight were increased significantly ($P < 0.05$) by more than 24.30 gr in fresh weight root and 1.9 gr in dry weight foliage compare to the untreated control (19.78 gr and 1.33 gr, respectively) (Table 3).

All EOs showed inhibition effects on two tested pathogens, however, sizes of inhibition zone were different depended on kind and concentration of oils. Thyme leaf EO possessed stronger antibacterial activity compared to the other tested oils, so that the highest inhibition zone was measured 34.8 mm on *R. solanacearum* (more than that of streptomycin- 22 mm) (Table 1).

After thyme, rosemary oil showed moderate antibacterial effect on both *R. solanacearum* and *P. carotovorum* (16.5 and 11.8 mm respectively) (Table 1 and 2). Negative controls did not show any antibacterial effect against the tested pathogens. In addition, *R. solanacearum* were resistant to standard antibiotic erythromycin. Antibacterial effect of the EOs was directly related to the concentrations, so higher diameters of zones were defined at 50, 75 and 100% concentration. MIC value of thyme oil exhibited more efficiency than the other tested EOs with 1 and 5 $\mu\text{g ml}^{-1}$ on *R. solanacearum* and *P. carotovorum* respectively. However, MIC and MBC values were seen very varied in different oils from 1 to 1000 $\mu\text{g ml}^{-1}$.

Results of fresh and dry weight showed significant differences between positive controls and treated-potatoes inoculated with *R. solanacearum* (Table 4). Fresh and dry root and foliage in wilted plants (positive control) were also reduced. Wilt incidence was 36.67% in treated contaminated potatoes which was significantly less than positive control (80.67%) (Table 4).

The average disease incidence on tomato is listed in Table 5. Based on these results, percentage of disease on plants treated by thyme (27%) was considerably less than that of the positive control (77.33%). In addition, there were significant differences between treated infected tomatoes and positive control samples on both dry and fresh weight (Table 5).

As shown in Table 6, the highest disease control was obtained by 64.73% in bacterial wilt on treated tomatoes, whereas thyme reduced the symptoms of wilt and rot on contaminated potatoes by 53.85% and 50.2%, respectively. The biomass of root and foliage of treated contaminated tomatoes with *R. solanacearum* were increased by 39.84% and 38.04%, respectively, while those of treated infected potatoes were increased by 23.25% and 22.30%, respectively (Table 6).

Table 3: Disease incidence of applying thyme oil on potatoes contaminated by *P. carotovorum* in greenhouse

Treatment	Disease incidence(%)	Fresh weight root(gr)	Fresh weight foliage (gr)	Dry weight root(gr)	Dry weight foliage(gr)
Positive control ^a	82.33a	19.78b	12.84b	1.18b	1.33b
Treated contaminated-plant	40.67b	24.30ab	15.51b	1.50b	1.95ab
Treated noncontaminated-plant ^b	0c	32.08a	18.98a	1.97a	2.14a
Negative control ^c	0c	32.09a	18.47a	1.91a	2.017a

^a untreated contaminated plant

^b vehicle [0.5% dimethyl sulfoxide (DMSO) + 0.1% Tween 80 + thyme oil 0.5% + water].

^c untreated non-contaminated plant

Values in the same column followed by the same letter are not significantly different at ($P < 0.05$) according to Duncan Multiple Range Test.

Table 4: Disease incidence of applying thyme oil on potatoes contaminated by *R. solanacearum* in greenhouse

Treatment	Disease incidence (%)	Fresh weight root (gr)	Fresh weight shoot (gr)	Dry weight root (gr)	Dry weight shoot (gr)
Positive control ^a	80.67a	14.47c	12.79c	1.31c	1.84b
Treated contaminated plant	36.67b	18.88b	16.55b	2.61b	2.69a
Treated non-contaminated plant ^b	0c	22.82a	23.55a	3.0a	3.26a
Negative control ^c	0c	23.08a	23.32a	3.08a	3.25a

^a untreated contaminated plant

^b vehicle [0.5% dimethyl sulfoxide (DMSO) + 0.1% Tween 80 + thyme oil 0.5% + water].

^c untreated non-contaminated plant

Values in the same columns followed by the same letters are not significantly different at ($P < 0.05$) according to Duncan Multiple Range Test.

Table 5: Disease incidence of applying thyme oil on tomatoes contaminated by *R. solanacearum* in greenhouse

Treatment	Disease incidence(%)	Fresh weight root(gr)	Fresh weight shoot(gr)	Dry weight root(gr)	Dry weight shoot(gr)
Positive control ^a	77.33 a	1.22c	1.98c	0.15b	0.29b
Treated contaminated plant	27b	2.03b	3.2b	0.32a	0.47a
Treated noncontaminated plant ^b	0c	2.57a	4.16a	0.40a	0.53a
Negative control ^c	0c	2.69a	4.19a	0.40a	0.54a

^a untreated contaminated plant

^b vehicle [0.5% dimethyl sulfoxide (DMSO) + 0.1% Tween 80 + thyme oil 0.5% + water].

^c untreated non-contaminated plant

Values in the same columns followed by the same letters are not significantly different at ($P < 0.05$) according to Duncan Multiple Range Test

IV. DISCUSSION

The results of current study showed that thyme oil had effective antibacterial effects against the tested bacteria in the culture media along with it reduced two dangerous antibacterial wilt and soft rot incidence on potato and tomato plants under natural conditions, afterward the greenhouse results confirmed those of *in vitro* assays.

Several reports confirm the efficiency of thyme on inhibition of phytopathogenic bacteria in greenhouse. Antibacterial activity of thyme was shown 69% against *Xanthomonas citri* pv. *citri* [17]. Also, Lucas *et al.* observed disease reduction against *Xanthomonas vesicatoria* with using thyme oil at concentration of 0.1% [18]. Recently, several researches were conducted on assaying different oils against *Ralstonia* and *Pectobacterium* which shows the importance of issue.

Table 6: Biocontrol efficiency and Biomass increase in bacterial contaminated plants which were treated with thyme oil in greenhouse

Disease	Biocontrol Efficiency	Biomass Increase Root	Biomass Increase Foliage
Soft rot	50.2a	18.55b	17.33b
Bacterial wilt	53.85a	23.25b	22.30b

Values in the same columns followed by the same letters are not significantly different at ($P < 0.05$) according to Duncan Multiple Range Test.

Values in percent

Jeong [19] used *Cymbopogon* sp. oil at concentration of 0.5% growth of *P. carotovorum* and observed complete inhibition activity. Vukovic [20] demonstrated that *Teucrium* sp. had antibacterial influence on *P. carotovorum*. Moreover, Biavati [21] represented antibacterial activity of *Satureja* sp. and *Thymbra* on *P. carotovorum*. Also, Vokou [22] demonstrated antibacterial effects of rosemary on infected-tubers with *P. carotovorum*. Ji [23] tested thymol on *Ralstonia* under field condition and observed suppression of bacterial wilt by treating with oil.

Results of other researches displayed that presence of thymol and carvacrol substances in some plants are responsible for their bactericidal activities [6]. *Thymus vulgaris* contains high quantities of thymol which potent, antibacterial activity of thyme oil. As the main mode of action of EOs is membrane structure disturbance and release of lipids to the cytoplasm, it makes them more permeable [24].

In our greenhouse tests, we observed a high reduction in disease occurred by the activity of thyme on both bacterial wilt and soft rot (64% and 50% respectively). Components of the oils may induce sort of plant systemic resistance which cause a reduction in disease incidence when the soil is drenched by the oil 48 h before it contaminated with bacteria species. It is documented that the plants had defense mechanisms against pathogen attacks, and some of these mechanisms are induced by biotic and non biotic materials. The resistance induced is distinguished by limitation of pathogen growth and suppression of disease symptoms progress [25].

V. CONCLUSION

In conclusion, the thyme oil has proven to be consistently efficient in the integrated control bacterial wilt and soft rot disease under greenhouse conditions. Even though other greenhouse and field studies should be undertaken to confirm the effectiveness of EOs under natural conditions and the dose of usage to obtain reasonable disease biocontrol efficiency and economic benefits.

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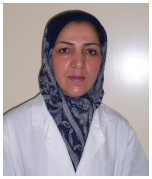
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