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INVESTIGATION OF POTANTIAL BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* F.SP. *RADICIS-LYCOPERSICI* AND *F. OXYSPORUM* F. SP. *LYCOPERSICI* BY ESSENTIAL OILS, PLANT EXTRACT AND CHEMICAL ELICITORS *IN VITRO*

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Abstract

Fusarium crown and root rot (caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, FORL) and *Fusarium* wilt (caused by *Fusarium oxysporum* f. sp. *lycopersici*, FOL) are the most important diseases on tomatoes. In the present study, the antimicrobial activity of 7 essential oil (cumin, thymus, lavandula, eucalyptus, rosemary, nigella, dill), 2 vinegars (apple and vine) and 2 chemical elicitors (BABA, JA) to FORL and FOL. Essential oils from cumin, thymus, nigella and dill showed the highest antagonistic effect against the pathogens. The inhibitory effects of vinegars and elicitors were not determined against FORL and FOL. BABA had no significant effect on mycelial growth with increasing concentration. However, the hyphal development was decreased with increasing concentration at 1000 ppm concentration. Mycelial growth of FORL and FOL was decreased by increasing JA at 100ppm concentration as well, but it was not inhibited completely.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the world's most important crop due to the high value of its fruits both for fresh market consumption. *Fusarium* crown and root rot (caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, FORL) and *Fusarium* wilt (caused by *Fusarium oxysporum* f. sp. *lycopersici*, FOL) are one of the most damaging diseases of tomato causing economic losses on plant in greenhouse in Turkey. It is very difficult to control with chemicals against *Fusarium* diseases in tomato. Although some resistant cultivars have been developed in the control of FOL, tomato cultivars resistant to the crown and root rot caused by FORL have not been developed yet (Fravel *et al*., 2003; Ozbay & Newman, 2004). However, the use of chemical has resulted in the development of fungicide resistant strains in the pathogen populations. The difficulties in controlling FORL and FOL have promoted scientists to search for biological alternatives methods (Browers & Locke, 2000). Some researchers investigated alternative control measures, varying from the application of essential oils, plant extracts and chemical elicitors (Browers and Locke, 2000; Lee *et al*., 2007; Hasem *et al*., 2010; Cohen *et al*., 2011). The essential oils, a group of plant derived compound are concentrated hydrophobic liquid including volatile aromatic compounds. The essential oils as diseases control agents have been used since it was known for their broad spectrum antimicrobial activity against plant pathogens. Many plant extracts and plant essential oils have been reported to be effective antimicrobials against soil-borne fungi (Browers & Locke 2000; Browers & Locke 2004, Dawar *et al*., 2007; Lee *et al*., 2007; Arici *et al*., 2011; Hasem *et al*., 2010). Infection of plants with a pathogen can enhance the plants resistance. Plants may be immunized against disease by abiotic or biotic elicitors (Goellner & Conrath 2008; Kuc, 2001; Walters *et al*., 2005, Cohen *et al*., 2011). The classical type of immunization is often referred to as systemic acquired resistance (SAR). DL-ß-amino-n-butyric acid and jasmonic acid are important signaling molecular in promoting resistance in plants. Active substances are taken from leaves/roots and act as a systemic signal in the plant (Jakab *et al*., 2001; Cohen 2002; Eschen-Lippold *et al*., 2010; Cohen *et al*., 2011).

In the present study, it was to investigate the antifungal activity of 6 essential oils, 2 vinegars and 2 chemical elicitors against the mycelial growth of FORL (*Fusarium oxysporium* Schl. f.sp. *radicis-lycopersici*) and FOL (*Fusarium oxysporium* Schl. f.sp. *lycopersici*) *In vitro*.

Material and Methods

Pathogen cultures: FORL (*Fusarium oxysporium* Schl. f.sp. *radicis-lycopersici*), FOL (*Fusarium oxysporium* Schl. f.sp. *lycopersici)* were cultured at 25°C on PDA medium.

Distillation of samples plant: The essential oils of plants (lavandula, eucalyptus, rosemary, cumin, thymus, nigella, dill) were hydro-distilled in a Clevenger-type apparatus for 3 h to obtain essential oil described in European Pharmacopoeia (1975). Essential oils of plants were dissolved in ethanol. Commercial apple and wine vinegars were used as plant extract in this study.

Chemical elicitors: DL-ß-amino-n-butyric acid (Fluka) and jasmonic acid (Sigma Aldrich) were used as elicitor. They were dissolved in water before used.

Antifungal activity: To determine the essential oils, plant extract and chemical elicitors effects on FORL and FOL different concentrations of DL-ß-amino-n-butyric acid (BABA) and jasmonic acid (JA) were tested. After sterilization of PDA media, essential oils, vinegars (Table 1) and chemical elicitors (Table 2) were added at different concentration into 20ml PDA and poured in Petri plates. Mycelial plugs (6mm in diameter) from the edges of each culture were incubated in the center of each PDA plate (9 cm diameter). Cultures were incubated in the dark at 26°C and 70% RH for 7-10 days. Mycelial growth was measured every day until control plates were completely colonized with mycelium. Replications were considered simultaneously for each concentration of samples. Only PDA was used as a control. All tests were repeated in triplicate with five replications.

Table 1. Treatment of essential oils and vinegars against FORL and FOL.

Application		Concentration (%)
Essential oil	Cumin (*Cuminum cyminum*)	0.5 1 2
	Thymus (*Thymus vulgaris*)	
	Lavandula (*Lavandula spica*)	
	Eucalyptus (*Eucalyptus globulus*)	
	Rosemary (*Rosmarinus officinalis*)	
	Nigella (*Nigella sativa*)	
	Dill (*Anethum graveolens)*	
Vinegars	Apple	5 10 20
	Wine	

Table 2. Treatment of chemical elicitor against FORL and FOL.

Chemical elicitor	Concentration (ppm)
B-Delta amino-n-butryric acid (BABA)	100 1000 2000
Jasmonic acid (JA)	25 50 100

Statistical analyses: All statistical analyses were carried out using SPSS 16 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences between means were determined by Duncan's pairwise comparison test at a level of $p<0.05$.

Results

Effect of essential oil on mycelial growth on FORL and FOL: Different essential oils exhibited different antifungal activities to both of the fungal species. There were significantly differences between treatment and concentration to essential oils. *In vitro*, essential oils extracted from cumin, thymus and dill showed the highest antagonistic effect against the FORL and FOL. The inhibitory effects of the essential oils on the mycelial growth of pathogens were observed with 2% of test compound rather than 1% for both of pathogens. The results of the antifungal tests revealed that the oils were inhibitory against all of the tested fungi. The mycelial growth rate of FORL for thymus was 0±0 compared with control 9±0.1 cm. Thyme oil, cumin, dill were the most effective antifungal compound to FORL and completely inhibited the growth of FORL at concentration of 1% and 2% dill and thymus, 1% cumin whereas eucalyptus, rosemary and nigella oils was the least effective among all the test compounds to FORL (Fig. 1). The highest mycelial growth rate (8.6±0.3 cm) was obtained with eucalyptus at 1% concentration compared with control 9±0.1cm (Table 3). Essential oil reduced mycelial growth of FORL about 5.6-100%.

Three concentrations of thymus, cumin and dill oils completely inhibited the growth of FOL. The mycelial growth rate of FOL for thymus, cumin and dill oil was 0±0 compared with control 9±0.1 cm. The lowest mycelial growth rate (2.2±0.1cm) was obtained with nigella at 2% concentration compared with control 9±0.1cm (Table 4). A non-significant activity was observed with lavandula, eucalyptus, rosemary, nigella oil to FOL. The mycelial growth of FOL was inhibited about 11.1-100%. It is interesting to note that cumin exhibited high antifungal activity against FOL but it was not found equally effective against FORL.

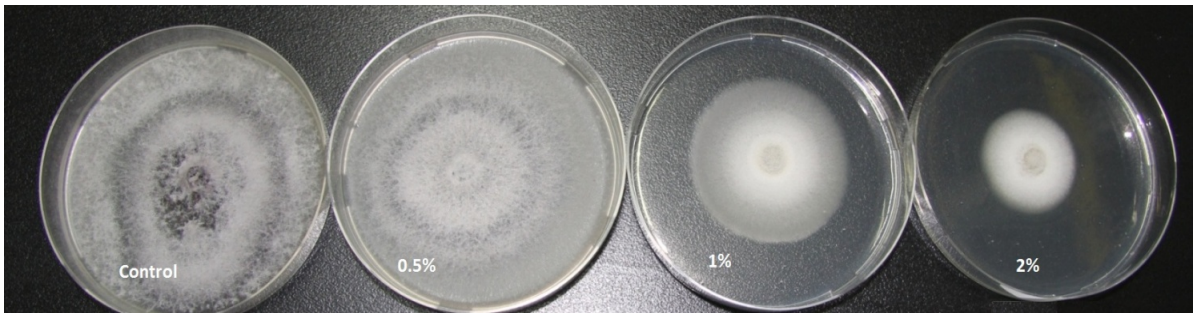

Fig. 1. Effect of nigella with different concentration to FORL.

Effect of vinegars on mycelial growth on FORL and FOL: At present study it has determined that vinegars presented weak activity to both of pathogens. The lowest mycelial growth rate (7.6±0.1 cm) was obtained with wine vinegar at 20% concentration, with apple vinegar 7.8±0.6 at 10% concentration for FOL compared with control 9±0.1 cm (Table 4). However there was no statistically effective to inhibit the growth of FORL (Table 3).The mycelial growth rate of FORL for apple vinegars was 8.2±0.1cm compared with control 9±0.1cm. Both of vinegars inhibited the mycelial growth of candidate pathogens about 4.4-17.8%.

Effect of chemical inducers on mycelial growth on FORL and FOL: *In vitro* test showed that BABA with 1000, 2000 ppm and JA with 50, 100 ppm reduced weakly of the mycelial growth on to PDA medium as compared to the control. Concentrations of 100 ppm BABA did not affect the mycelial growth of FORL with 8.9 cm (Table 5) and FOL with 8.5 cm (Table 6). The

mycelia growth was decreased with increasing concentration occurred at BABA with 1000 ppm concentration. The mycelial growth was found 8cm at 2000 ppm, and 9.1 cm in control. BABA with 2000 ppm inhibited about 8-12% mycelial growth of FORL and FOL. Similar result was observed in the application of JA. Mycelial growth of FORL and FOL was decreased by increasing JA concentration as well but JA did not inhibit the mycelial growth of candidate pathogens completely. It reduced the mycelial growth with concentration up to 50 ppm. The mycelial growth rate was found 7.9cm for FORL, 7.7 cm for FOL at 100 ppm. Mycelial growth of candidate fungi was decreased to 12-14% at 100 ppm of FA concentration.

Discussion

At present study, essential oils extracted from cumin, thymus and dill showed the highest antagonistic effect against FORL and FOL. The antifungal activity of thymus oil was well established against fungi such as *B. cinerea* (Tripathi & Dubey, 2008; Barrera-Necha *et al*., 2009), *Rhizoctonia solani, Pythium ultimum, Fusarium solani,* and *Colletotrichum lindemuthianum* (Zambonelli *et al*., 1996; Arici *et al*., 2011), *Fusarium* spp. (Hasem *et al*., 2010). It was reported that cumin, thymus and rosemary inhibited the mycelial growth of *Fusarium oxysporum* f.sp. *cicer* (Pawar & Thaker, 2007).

There are several studies to reveal the potential of essential oils as antifungal agents (Ko *et al*., 2003; Oxenham *et al*., 2005; Dawar *et al*., 2007; Lee *et al*., 2007; Barera-Necha *et al*., 2009; Hasem *et al*., 2010). In our experiment, lavandula and eucalyptus oil had no activities, whereas rosemary oil presented weak activity to both of pathogens. Mycelial growth of both pathogens was quite poor. Barrera-Necha *et al*., (2009) reported that eucalyptus also had no activity on mycelial growth of *F. oxyporium* f.sp. *gladiola*, although Daferera *et al.,* (2003) found that lavandula, rosemary oil were also fungi toxic on mycelial growth of *B. cinerea* and *Fusarium* sp., but at higher concentrations. In present study, these concentrations (1-2%) could partially inhibit spore germination and germ tube elongation. The high concentration of the essential oils could inhibit mycelial growth of FORL and FOL completely *In vitro*. It should be investigated the effective concentration to FORL and FOL in the future studies.

Table 3. Growth inhibition of FORL treated with essential oils and vinegars.

Essential oil/vinegar	Concentration (%)	Colony diameter (cm)* (FORL)	% Effect
Cumin	0.5	**4.8 ± 1.2 *de*	46.7
	1	4.5 ± 1.9 *d*	50.0
	2	0 ± 0 *a*	100.0
Thymus	0.5	0 ± 0 *a*	100.0
	1	0 ± 0 *a*	100.0
	2	0 ± 0 *a*	100.0
Lavandula	0.5	7.9 ± 0.2 *gh*	12.2
	1	7.3 ± 0.6 *g*	18.9
	2	5.9 ± 1.5 *f*	34.4
Eucalyptus	0.5	8.6 ± 0.3 *hi*	4.4
	1	8.4 ± 0.1 *hi*	6.7
	2	8.5 ± 0.2 *hi*	5.6
Rosemary	0.5	7.8 ± 0.3 *gh*	13.3
	1	7.3 ± 0.3 *g*	18.9
	2	5.9 ± 1.3 *ef*	34.4
Nigella	0.5	8.1 ± 0.3 *ghi*	10.0
	1	3.2 ± 0.3 *c*	64.4
	2	2.2 ± 0.2 *b*	75.6
Dill	0.5	0 ± 0 *a*	100.0
	1	0 ± 0 *a*	100.0
	2	0 ± 0 *a*	100.0
Wine vinegar	5	8.5 ± 0 *hi*	5.6
	10	8.2 ± 0 *hi*	4.4
	20	8.3 ± 0.1 *hi*	7.8
Apple vinegar	5	8.5 ± 0 *hi*	5.6
	10	8.4 ± 0 *hi*	6.7
	20	8.2 ± 0.1 *hi*	8.9
Control	-	9 ± 0.1 *I*	-

*Disc diameter of 6mm was subtracted from colony diameter

**Different letters are statistically different at $p<0.05$, according to the Duncan's pair wise comparison test

Table 4. Growth inhibition of FOL treated with essential oils and vinegars.

Essential oil/vinegar	Concentration (%)	Colony diameter (cm)* (FORL)	% Effect
Cumin	0.5	**0 ± 0 *a*	100.0
	1	0 ± 0 *a*	100.0
	2	0 ± 0 *a*	100.0
Thymus	0.5	0 ± 0 *a*	100.0
	1	0 ± 0 *a*	100.0
	2	0 ± 0 *a*	100.0
Lavandula	0.5	7.2 ± 0.7 *hi*	20.0
	1	7.7 ± 0.6 *ijk*	14.4
	2	5.6 ± 0.1 *e*	37.8
Eucalyptus	0.5	8.0 ± 0.4*jklm*	11.1
	1	7.8 ± 0.6 *ıik*	13.3
	2	7.7 ± 0.4 *ıik*	14.4
Rosemary	0.5	6.9 ± 0.4 *gh*	23.3
	1	6.5 ± 0.5 *fg*	27.8
	2	3.6 ± 0.8 *c*	60.0
Nigella	0.5	6.0 ± 0.1 *ef*	33.3
	1	4.6 ± 0.8 *d*	48.9
	2	2.2 ± 0.1 *b*	75.6
Dill	0.5	0 ± 0 *a*	100.0
	1	0 ± 0 *a*	100.0
	2	0 ± 0 *a*	100.0
Wine vinegar	5	7.7 ± 0.4*ijk*	14.4
	10	7.9 ± 0.2*jklm*	12.0
	20	7.6 ± 0.1*ijk*	15.6
Apple vinegar	5	7.4 ± 0.2*hij*	17.8
	10	7.8 ± 0.6 *ijk*	13.3
	20	8.2 ± 0.1 *hi*	8.9
Control	-	9 ± 0.1 *k*	-

*Disc diameter of 6mm was subtracted from colony diameter.
**Different letters are statistically different at p<0.05, according to the Duncan's pair wise comparison test

Table 5. Growth inhibition of FORL treated with chemical elicitors.

Chemical elictor	Concentration (%)	Colony diameter (cm)* (FORL)	% Effect
B-Delta amino-n-butryric acid (BABA)	100	**8.9 ± 0 b	1
	1000	8.5 ± 0.7 *ab*	6
	2000	8.3 ± 0.2 *ab*	8
Jasmonic acid (JA)	25	8.3 ± 0.2 *a*	8
	50	8.2 ± 0.2 *ab*	9
	100	7.9 ± 0.9 *a*	12
Control		9 ± 0.1 *b*	-

*Disc diameter of 6mm was substracted from colony diameter
**Different letters are statistically different at p<0.05, according to the Duncan's pair wise comparison test

Table 6. Growth inhibition of FOL treated with chemical elicitors.

Chemical elictor	Concentration (%)	Colony diameter (cm)* (FOL)	% Effect
B-Delta amino-n-butryric acid (BABA)	100	**8.5 ± 0.3 cde	6
	1000	8.5 ± 0.3 *ab*	6
	2000	7.9 ± 0.6 *ab*	12
Jasmonic acid (JA)	25	8.2 ± 0.2 *bcd*	9
	50	8.0 ± 0.2 *ab*	11
	100	7.7 ± 0.9 *a*	14
Control		9 ± 0.1 *e*	0

*Disc diameter of 6mm was substracted from colony diameter
**Different letters are statistically different at p<0.05, according to the Duncan's pair wise comparison test

Some researchers reported that several formulated botanical extracts were shown to effectively reduce soil populations. The antifungal activity of plant extract was reported against fungi such as *F. oxysporum* (Browers & Locke 2000), *Phytophthora nicotianae* (Browers & Locke 2004). In our study we determinated that vinegars presented weak activity to both of pathogens. At this concentration, the extract may not have inhibited the pathogen. The concentration of vinegars up to 20% may have effected to inhibit the mycelial growth of FOL and FORL sufficiently.

In the present study, BABA with 1000, 2000ppm reduced weakly the mycelial growth on to PDA medium. BABA had no effect on the mean mycelial growth of FORL and FOL between 100-1000 ppm concentrations. Arici & Dehne (2007) reported that BABA with 10000 ppm reduced about 10-15% mycelial growth of *P. infestans* and BION with 1000 ppm reduced about 10-15% of the mycelial growth on to V8-juice medium. Similarly, JA did not inhibit the mycelial development of FORL and FOL *In vitro.* Cohen *et al.,* (1994) reported that JA at 4.8mmol/ml did not inhibit the fungal mycelial growth of *P. infestans* in agar cultures. In some studies reported that although chemical elicitors had no effectiveness the development of mycelial growth of pathogens, they prevented the growth of pathogen in plants by promoting plant resistance against diseases following applications (Cohen & Gisi 1994; Cohen *et al*., 1994; El-Khallal, 2007; Cohen *et al*., 2010).

BABA and JA induce local and systemic resistance against disease in numerous plant species. El-Khallal (2007) reported that jasmonic acid induced the systemic acquired resistance in tomato plants against *Fusarium* wilt disease. Cohen *et al*., 2011 showed that preventive application of BABA to lettuce (*Lactuca sativa*) plants induced resistance against downy mildew caused by the oomycete *Bremia lactucae.*

Conclusion

The difficulties in controlling FORL and FOL have promoted scientists to search for biological alternatives. Essential oil, plant extract and chemical elicitor (BABA, JA) have been widely investigated for the control of disease of agricultural crops. In present study essential oils from cumin, thyme and nigella and dill showed the highest antagonistic effect against the pathogens, whereas wine and apple vinegars did not inhibit the growth of FORL and FOL. BABA and JA exhibited no fungicidal activity against FORL and FOL *In vitro*. Therefor they induced resistance against diseases. Induced resistance to plant diseases has been a method used as an alternative to the fungicides against plant pathogens in recent years. BABA and JA should require to be investigated against FORL and FOL *In vivo.*

In conclusion, essential oils showed the antifungal activities against phytopathogenic fungi *In vitro* and have the potential to be used as antifungal agents for the control of FORL and FOL on tomato plants. However, for the development of essential oils as potential antifungal agents, further studies are required to evaluate phytotoxicity of essential oils for application on plants and sensory quality of treated fruits and vegetables. If they inhibit the growth of pathogens in the field conditions, they can be used as alternative control measures and an environmentally safe to chemical control.

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