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ESSENTIAL OILS AND HOMEMADE FUNGICIDES AGAINST *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* TOMATO PATHOGEN

Andreea-Alexandra TIMOFTE, Andreea-Mihaela FLOREA, Florin Daniel LIPȘA, Eugen ULEA

„Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi, 3 Mihail Sadoveanu Alley, 700490, Romania

Corresponding author email: aalexandra.timofte@gmail.com

Abstract

The destructive disease of tomato worldwide, fusarium wilt, is caused by Fusarium oxysporum f. sp. lycopersici (Sacc) W. C. Synder & H. N. Hans., a vascular wilt pathogen. The fungus affects greenhouse and field grown tomatoes in warm vegetable production areas. Yellowed leaves and wilted plants with low or absent crop yield are the main symptoms of the disease. In this paper we have analyzed possible substitutes to synthetic fungicides in controlling the pathogen. Since the potential of essential oils as antimicrobial agents is well established and farmers around the world already use traditional recipes, we have decided to test a fair amount of essential oils and four homemade fungicides against the growth of Fusarium oxysporum f. sp. lycopersici mycelium. For the experiment, 1 ml of essential oil serial dilutions of 0.1%, 1% and 10% were used along with the most used worldwide homemade fungicides. Fungal growth measurements were taken every 24h for 13 days, until no fungal growth was registered. Results show that 0.1% essential oils concentration had no inhibitory effect, while at 1% oil concentration only oregano essential oil was 61% effective. At 10% cinnamon, cloves, thyme, oregano and lemon essential oils showed cidal effects.

Key words*: essential oils, fusarium wilt, in vitro, tomato disease.*

INTRODUCTION

The unifying principle behind most supporters of organic farming is the belief that soil health is extremely important for life and is the only sustainable way of cultivating land and creating a safe future for humanity.

The use of biopesticides has been possible since the 1960s, but has only increased recently due to society's awareness of the issues of intensive use of chemicals such as human health, soil pollution and water resources, such as and the resistance acquired by pests and pathogens. Biocontrol products are used in both conventional and organic farming.

Tomatoes (*Lycopersicon esculentum* Mill.) are highly susceptible to pathogens, such as fungi, viruses, bacteria and nematodes, which cause severe yield losses (Barone and Frusciante, 2007).

The destructive disease of tomato worldwide, fusarium wilt is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) W. C. Synder & H. N. Hans., a vascular wilt pathogen. The fungus affects greenhouse and field grown tomatoes in warm vegetable production areas. Yellowed leaves and wilted plants with low or absent crop yield are the main symptoms of the disease (Sudhamoy et al., 2009). The disease may lead to a 30 to 40% yield loss and it can even go up to 80% under favorable climate conditions (Kirankumar et al., 2008).

The pathogen enters the root and further into the vascular tissue. The xylem vessels are colonized by mycelium and conidia. As a result, the vessels are clogged and plants suffer of severe water stress, leading to the characteristic wilt symptoms (Beckman, 1987). There are three physiological pathogen races (1, 2 and 3) known to affect tomato cultivars (Kawabe et al., 2005).

MATERIALS AND METHODS

The antifungal activity of 22 commercially purchased essential oils, i.e. anise (*Pimpinella anisum* L.), basil (*Ocimum basilicum* L.), Indian frankincense (*Boswellia serrata* T.), cinnamon (*Cinnamomum aromaticum* L.), camphor tree (*Cinnamomum camphora* L.), lemongrass (*Cymbopogon winterianus* L.), cloves (*Syzygium aromaticum* L.), coriander (*Coriandrum sativum* L.), May Chang (*Litsea cubeba*), fennel (*Foeniculum vulgare* M.), oil grass (*Cymbopogon citratus* DC.), lavender (*Lavandula angustifolia* Mill.), tea tree (*Melaleuca viridiflora* Sol.), orange (*Citrus* x *sinensis* L.), palmarosa (*Cymbopogon martinii* Roxb.), turmeric (*Curcuma longa*), rosemary (*Rosmarinus officinalis* L.), clary sage (*Salvia sclarea* L.), spearmint (*Mentha spicata* L.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.) and lemon (*Citrus limon* L.), along with homemade fungicides based on baking soda, garlic and hydrogen perodixe were investigated against *F. oxysporum* f. sp. *lycopersici.*

For the homemade fungicides, we have selected the most used recipes by international farmers: mix 5 tablespoons of baking soda with 1 teaspoon of liquid soap in 1 gallon of water (B1); mix 1 tablespoon of baking soda with 1 teaspoon of castor oil in 1 gallon of water (B2); chop 100 g of garlic cloves in 1 L of water Us (Ga); hydrogen peroxide 3% AO (HP).

For the experiment, 1 mL of EO dilution or homemade fungicide was pipetted in each Petri dish (85mm), after which 17 mL of potato dextrose agar (PDA, Scharlau, Spain) were added at temperature of 50ºC, to avoid volatilizing or denaturing the aromatic compounds in the oils, then the dishes were stirred for 20s. EO concentrations of 0.1%, 1% and 10% were expressed using Percent Composition by Mass (%), in which the mass of the solute is divided by the mass of the solution (mass of solute plus mass of solvent), then multiplied by 100. Media was allowed to cool and solidify. After 2 h, a 7 mm mycelial plug of *F. oxysporum* f. sp. *lycopersici* was centered onto each Petri dish. The *F. oxysporum* f. sp. *lycopersici* cores were taken from the edge of individual 14 days old colonies. All Petri dishes were left inside the laminar-flow hood for 24 h then stored inverted so that water would not condense on the agar surface. Dishes were incubated in the dark inside a 28ºC germination chamber. A total of 66 dishes were used per replication, with 3 replications. Control plates were included in each replication: 3 PDA media plates inoculated with the pathogen, to determine the viability and growth.

Fungal growth measurements were taken every 24 h for 13 days, until no fungal growth was registered. One Way Within Subjects ANOVA was used to determine the effect of treatments on each concentration on growth measurements. Statistical analysis was performed with the IBM SPSS Amos v20.

RESULTS AND DISCUSSIONS

The maximum growth of *F. oxysporum* f. sp. *lycopersici* at the end of the 13 days experiment is outlined in Figure 1.

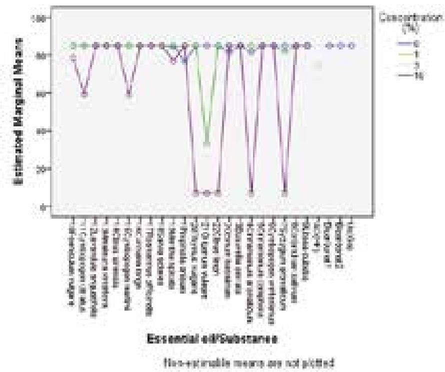

Figure 1. Effect of EOs and homemade fungicides on growth of *Fusarium oxysporum* f. sp. *lycopersici*, at different concentrations (original)

The oregano (*Origanum vulgare*) EO is the only oil that has the most aggressive effect on the growth of the fungus: both at 1% and 10% concentration, the mycelium was stopped from developing (Figure 2); on the other hand, the 0.1% oil concentration did not prevent growth in any way. The main components of the oil are carvacrol, thymol, p-cymene, cis-omen, caryophyllene and linalool.

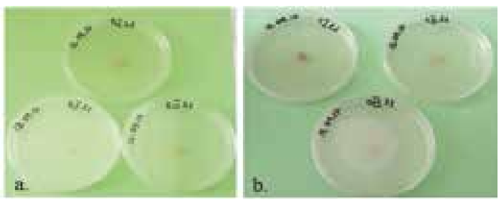

Figure 2. Cidal effect of oregano EO on growth of *Fusarium oxysporum* f. sp. *lycopersici*, at 1% (a) and 10% (b) oil concentrations (original)

There are a number of essential oils that did not allow the fungus any growth at 10% oil concentration, i.e. cinnamon (*Cinnamomum aromaticum*), cloves (*Syzygium aromaticum*), thyme (*Thymus vulgaris*) (Figures 3 and 4), and lemon (*Citratus limon*) EOs.

Figure 3. Cidal effect of cinnamon (*Cinnamomum aromaticum*) (a), cloves (*Syzygium aromaticum*) (b) and thyme (*Thymus vulgaris*) (c) EOs on *Fusarium oxysporum* f. sp. *lycopersici*, 10% oil concentration (original)

Cinnamon and cloves EOs have eugenol and eugenol acetate. As distinct elements, cinnamon oil also contains cinnamic aldehyde and benzyl benzoate, and cloves oil also has iso-eugenol and caryophyllene. The α-pinene, camphen, β-pinene, α-terpinene and linalool are found to be both in thyme and lemon EOs.

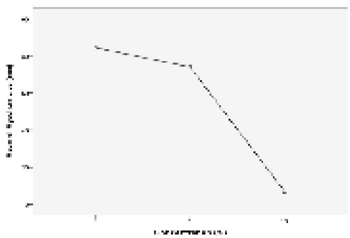

Figure 4. Average size of *Fusarium oxysporum* f. sp. *lycopersici* mycelium under treatment with cinnamon (*Cinnamomum aromaticum*), cloves (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) EOs at 0.1%, 1% and 10% concentrations (original)

Effects of EOs treatments on growth of *F. oxysporum* f. sp. *lycopersici* are significant, Wilks'Lambda = 0.71, $F(2, 20) = 3.95$, $p = 0.036$ (Table 1).

Table 1. Multivariate Tests[a] - *Fusarium oxysporum* f. sp. *lycopersici*

Effect		Value	F	Hypothesis df	Error df	Sig.
Factor 1	Pillai's Trace	.283	3.954[b]	2.000	20.000	.036
	Wilks' Lambda	.717	3.954[b]	2.000	20.000	.036
	Hotelling's Trace	.395	3.954[b]	2.000	20.000	.036
	Roy's Largest Root	.395	3.954[b]	2.000	20.000	.036

a. Design: Intercept
Within Subjects Design: factor1
b. Exact statistic

Since the Sig. value for Wilks' Lambda variation is lower than 0.05, there is a significant difference between the effect of each oil concentration.

The Paired T-Test (Table 2) was further used to compare the *post hoc* effect of each oil concentration on the fungus development.

Table 2. Paired Samples Test - *Fusarium oxysporum* f. sp. lycopersici

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
concentration=0.1% - concentration=1%	-2.227	13.349	2.846	-8.146	3.691	-.783	21	.443
concentration=0.1% - concentration=10%	-25.455	41.977	8.950	-44.066	-6.843	-2.844	21	.010
concentration=1% - concentration=10%	-23.227	38.295	8.164	-40.206	-6.248	-2.845	21	.010

The T-test indicates there are significant differences between 0.1% - 10%, as well as between 1% - 10% EOs concentrations, since the Sig. value, also known as the p value, is lower than 0.017.

CONCLUSIONS

All the EOs showed low influences at 0.1% concentrations, up to 14%.

The oregano (*Origanum vulgare*) EO treatment demonstrated a 61% efficacy on the *F. oxysporum* f. sp. *lycopersici* mycelium at 1% oil concentration.

The fungus was influenced by a reduced number of EOs at 10% concentration, but they all demonstrated a cidal effect, i.e. cinnamon (*Cinnamomum aromaticum*), cloves (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and lemon (*Citrus limon*).

Usually, the effects are seen in a time- and dose-dependent manner; higher concentrations cause severe effects more rapidly.

As the present paper showed, the oil concentrations necessary to kill *F. oxysporum* f. sp. *lycopersici* are most of the times much higher than those required to inhibit its growth.

REFERENCES

Barone A., Frusciante L., 2007. Marker-assisted selection: current status and future perspectives in crops, livestock, forestry and fish (eds Guimaraes E. et al.) Ch. 9, 151-164 (Agriculture and Consumer Protection Dept.).

Beckman C.H., 1987. The nature of wilt diseases of plants 175-176 (American Phytopathological Society).

Hariprasad P., Navya H.M., Chandra Nayaka S., Niranjana S.R., 2009. Advantage of PSIRB over PSRB and IRB to improve plant health of tomato. Biol. Cont. 50, 307-316.

Kawabe M. et al., 2005. Three evolutionary lineages of tomato wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici*, based on sequences of IGS, MAT1, and pg1, are each composed of isolates of a single mating type and a single or closely related vegetative compatibility group. J. Gen. Plant Pathol. 71, 263-272.

Kirankumar R., Jagadeesh K.S., Krishnaraj P.U., Patil M.S., 2008. Enhanced growth promotion of Tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of Tobacco Mosaic Virus pathogen. J. Agric. Sci. 21, 309-311.

Sudhamoy M., Nitupama M., Adinpunya M., 2009. Salicylic acid induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiol. Biochem. 47, 642-649.