

ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AND HOMEMADE FUNGICIDES AGAINST *PASSALORA FULVA*

Andreea–Alexandra TIMOFTE¹, Florin Daniel LIPȘA¹, Andreea–Mihaela FLOREA¹, Eugen ULEA¹

e-mail: aalexandra.timofte@gmail.com

Abstract

Passalora fulva is a pathogen which causes the disease on tomato known as the tomato leaf mold. In the greenhouses, this disease causes big problems during the fall, in early winter and spring, due to the high relative humidity of air and the temperature. The development of the disease is quick, moving from lower to upper leaves so the controlling must be done as soon as possible. The antifungal activity of medicinal plants bioactive compounds has gained a lot of attention within the scientific community. The main factor is the growing problem of multidrug resistance among pathogenic fungi. In addition, medicinal plant oils are the promising sources of antifungal drugs. Based on these facts, the present study emphasizes the importance of plants EOs as an alternative against pathogenic fungi causing tomato leaf mould. The spread of pesticide resistant pathogens is one of the most severe threats to successful treatment of microbial diseases. Renewed recent interest towards EOs utilization has been attributed to several factors, including a general revival in the appeal of ‘natural’ products, the desire for antimicrobial compounds with considerably better safety and toxicity profiles, and more importantly, the need for alternative ways to conventional antimicrobial, since they show reduced susceptibility to many major pathogens. In this paper we have analyzed possible substitutes to synthetic pesticides in controlling the fungus. Since the potential of essential oils (EOs) 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 homemade fungicides against the growth of *Passalora fulva* mycelium. We have discovered cloves EO kills the fungus at any tested concentration. The next EOs with strong effects are cinnamon, lemongrass, palmarosa, oregano and oil grass. The rest of EOs have shown a medium inhibitory effect.

Key words: essential oil, antifungal activity, *Passalora*, *in vitro*, mycelial growth

Passalora fulva Braun *et al*, 2003 (syn. *Cladosporium fulvum* Cooke 1878; *Fulvia fulva* Cooke Cif., 1954) is the causal organism of the fungal disease tomato leaf mould. Foliage is mainly the only tissue affected by the fungus, although occasionally other plant parts such as stems, blossoms, petioles and fruits are attacked (Butler E.J. and Jones S.G., 1949; Jones J.B. *et al*, 1997). Initial disease symptoms occur at least one week after the infection, showing pale green or yellowish diffuse lesions on the upper leaf surface, which later enlarge and turn into distinctive yellow spots (Tsitsigiannis D.I. *et al*, 2008). Under severe conditions, these lesions unite leading the leaves to curling, wilting and dropping; green or mature fruit can develop a dark wrinkled spot at the stem end, with damaged area as much as one third of the fruit

(Jones J.B. *et al*, 1991; Panagopoulos C.G., 2000; Thomma B.P.H.J. *et al*, 2005).

The potential of essential oils (EOs) as antimicrobial agents is well established. EOs are mixtures of volatile secondary metabolites and also exhibit activity against fungi, activity that is becoming increasingly well described (Jansen A.M. *et al*, 1987). A wide range of human, animal and agricultural fungal pathogens have been shown *in vitro* to be inhibited and/or killed by essential oils, heightening interest in their therapeutic or industrial application.

A series of EOs and homemade fungicides were evaluated for their efficacy as potential fungicides against *Passalora fulva*. Agar-dilution method was used in which 1 mL of serial dilution was inoculated at concentrations of 0.1%, 1% and 10%. A 7 mm mycelial plug was placed in the

¹ “Ion Ionescu de la Brad” University of Agricultural Sciences and Veterinary Medicine, Iasi

center of each 85 mm Petri dish and after 24h the mycelium size has been measured daily for a period of 26 days until no growth was recorded. Not all EOs were found to kill or inhibit the growth of *Passalora fulva* mycelium.

The aim of this paper was to investigate alternatives to synthetic fungicides currently used in the control of *Passalora fulva* pathogen, the causal agent of tomato leaf mould. In view of these considerations, the present work examined the antifungal activity of 22 EOs and 4 homemade fungicides, underlining those which showed the best antifungal profile, information which will be used later for an *in vivo* study against *Passalora fulva* in greenhouse conditions.

MATERIAL AND METHOD

Twenty-two EOs purchased from Naturela Ltd, Romania were used in this study for testing *in vitro* the fungitoxicity on *Passalora fulva*, 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 peroxide were investigated against *P. fulva*.

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 (BS1); mix 1 tablespoon of baking soda with 1 teaspoon of castor oil in 1 gallon of water (BS2); 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), then 17 mL of potato dextrose agar (PDA, Scharlau, Spain) were added at temperature of 50°C, to avoid volatilizing or denaturing the aromatic compounds of the oils, after which 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 2h, a 7 mm mycelial plug of *P. fulva* (isolated from infected tomato leaves) was centered onto each Petri dish. The *P. fulva* cores were taken from the edge of individual 14 days old colonies. All Petri dishes were left inside the laminar-flow hood for 24h then stored inverted so that water would not condense on the agar surface. Dishes were incubated in the dark inside a 27°C 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 24h for a period of 26 days, until no fungal growth was registered. Two-Way ANOVA was used to determine the effect of treatments per concentration on growth measurements. Statistical analysis was performed using the IBM SPSS Amos v20 software.

RESULTS AND DISCUSSIONS

In this study the antifungal activity of oils listed above was evaluated by measuring the mycelium growth of *P. fulva*.

Figure 1 shows the growth of mycelium size in mm by the end of the 26 day recording period. The most satisfying result of EO activity was obtained when using cloves EO (*Syzygium aromaticum* L.): it showed full cidal effect at all concentrations. The major components of cloves EO are: eugenol, eugenol acetate, iso-eugenol and caryophyllene. Eugenol is present in concentrations of 80 – 90% in clove bud oil and at 82 – 88% in clove leaf oil (Barnes J. *et al*, 2007).

At 1% and 10% concentrations, cinnamon (*Cinnamomum aromaticum*) EO has proven also a cidal effect on the fungus. Eugenol and eugenol acetate are found in its composition which proves once again the effectiveness of this phenylpropene.

Citronellal, geraniol, limonene and citral are the common elements found in lemongrass (*Cymbopogon winterianus* L.) and oil grass (*Cymbopogon citratus* DC.) EOs which killed the fungus at concentrations of 1% and 10% and inhibited it at 0.1%. Geraniol and limonene are found in palmarosa (*Cymbopogon martinii* Roxb.) EO also, along with myrcene, which is found in oil grass EO as well and revealed the same effect: killed the fungus at 1% and 10% and inhibited its growth at 0.1%, up to maximum 38 mm diameter. Turmeric (*Curcuma longa*) with its major component turmerone killed the fungus at 10% and grew up to 74 mm at the other concentrations

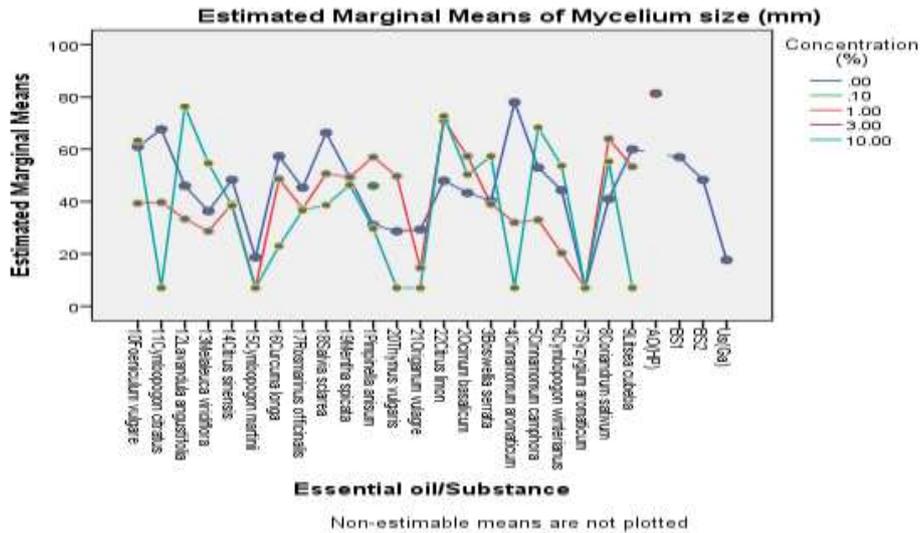


Figure 1 Effect of all EOs and homemade fungicides on *Passalora fulva* mycelium size at different concentrations (original)

Thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare* L.) EOs have a similar compositions, sharing p-cymene, linalool, β -caryophyllene, thymol and carvacrol. Oregano oil killed the fungus at 1% and 10%, while thyme oil only at 10%. At other concentrations, maximum growth was of 63 mm when treated with thyme oil and 52 mm when oregano oil was used.

All the others EOs have shown a medium inhibitory effect, noting the fact that cinnamon (*Cinnamomum aromaticum* L.) and clary sage (*Salvia sclarea* L.) EOs allowed the fungus to grow up to 82 mm.

Regarding the homemade fungicides, the baking soda recipes proved to be average in constraining the fungal growth, while the garlic recipe allowed the fungus to grow up to 16, 17 and 20 mm in diameter. The hydrogen peroxide 3% showed no effect, the mycelium growing to 80 and 82 mm.

From a time-series point of view, the evolution of *P. fulva* mycelium was distinct under treatment with spearmint (*Mentha spicata* L.) (figure 2), tea tree (*Melaleuca viridiflora* Sol.) (figure 3) and lemongrass (*Cymbopogon winterianus* L.) (figure 4) EOs.

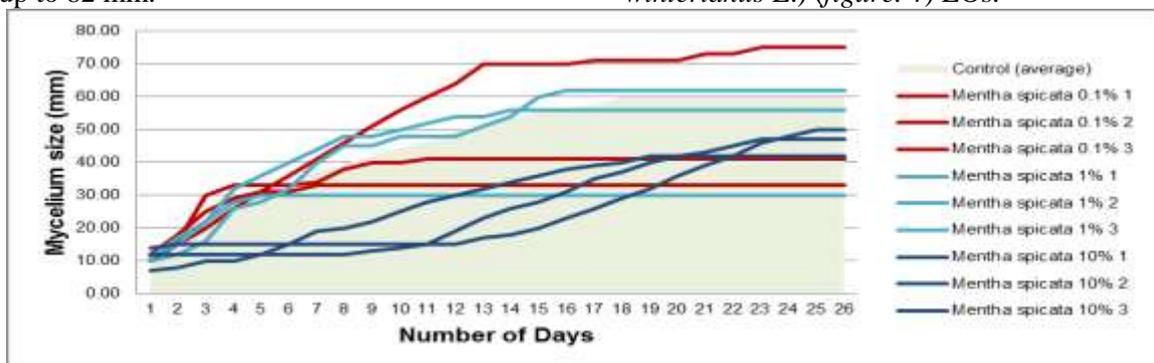


Figure 2 Effect of *Mentha spicata* EO on *Passalora fulva* mycelium size at different concentrations (original)

The mycelium showed no growth when spearmint (*Mentha spicata* L.) EO was used at 10% concentration for 8 to 11 days since inoculation. After the constituents were transformed in less toxic compounds, the growth reached 50 mm in diameter. At lower concentrations, the size was of 30 to 74 mm.

At 10% tea tree (*Melaleuca viridiflora* Sol.) EO concentration, in 2 trials the mycelium showed no sign of growth for 11 – 12 days, after which it has grown at an accelerated pace to 75 and 80 mm. At the other concentrations, the EO developed in the first 3 to 7 days to medium sizes ranging from 22 mm to 56 mm



Figure 3 Effect of *Melaleuca viridiflora* EO on *Passalora fulva* mycelium size at different concentrations (original)

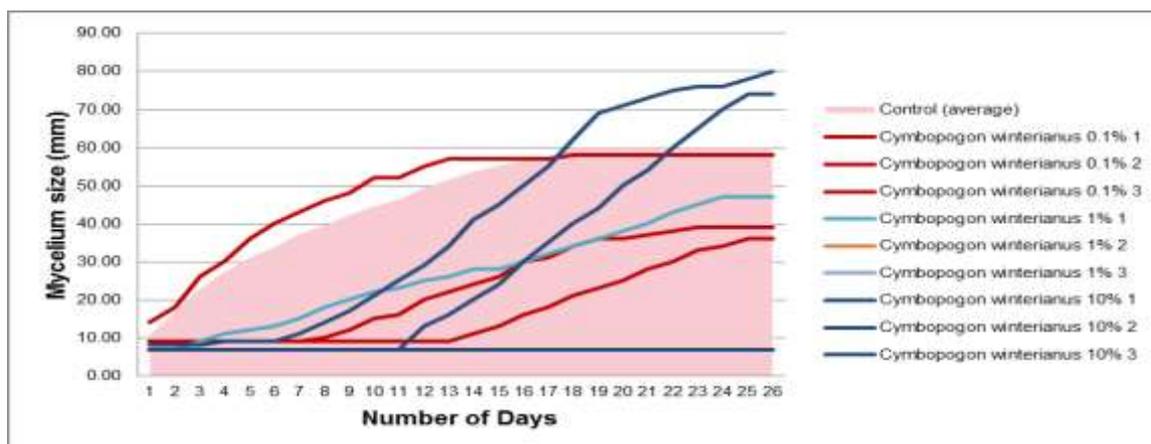


Figure 4 Effect of *Cymbopogon winterianus* EO on *Passalora fulva* mycelium size at different concentrations (original)

Lemongrass (*Cymbopogon winterianus* L.) EO at 0.1% stopped the fungus from growing for 7 – 13 days in two of the three repetitions, reaching after that a size of 39 mm. At 1% concentration, the fungus was able to grow only in one of the repetitions, up to 47 mm. At 10%, the mycelium had a very chaotic evolution. In one repetition it slowly grew from day 2, in another one it did not grow for 11 days and in the last one the fungus did not develop at all.

CONCLUSIONS

The only oil which has shown full cidal effect at all the tested concentrations is cloves (*Syzygium aromaticum* L.) EO. At 1% and 10% concentrations, cinnamon (*Cinnamomum aromaticum*), lemongrass (*Cymbopogon winterianus* L.), palmarosa (*Cymbopogon martinii* Roxb.), oregano (*Origanum vulgare* L.) and oil grass (*Cymbopogon citratus* DC.) EOs killed the fungus. Thyme (*Thymus vulgaris* L.) EO was capable of killing the fungus only at 10% concentration. All the others EOs have shown a medium inhibitory effect, except for cinnamon (*Cinnamomum aromaticum* L.) and clary sage (*Salvia sclarea* L.) EOs which allowed the fungus to grow up to 82 mm.

Chemicals derived from natural plants such as essential oils should be considered as potential alternative pesticides because consumer concerns today are focusing on the general toxicity of synthetic chemicals. However, it is important to develop a better understanding of the biological activities of essential oils for use to prevent various tomato diseases without relying on synthetic chemicals.

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