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EVALUATION OF THE SENSITIVITY OF SELECTED PATHOGENIC FUNGI TO HERBAL ADDITIVES AND THE PRESENCE OF AN ANTAGONISTIC FUNGUS IN *IN VITRO* CONDITION

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Abstract

In the counteract pathogenic fungi, alternative methods of plant protection are becoming more and more important, using both antagonistic microorganisms and vegetable active biological substances. The purpose of the work was to determine the effect of dried three plants on the growth and development of 8 species of phytopathogenic fungi, economically important (*Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. oxysporum, F. solani, Monilinia fructigena, Sclerotinia sclerotiorum, Trichothecium roseum*). For fungi cultures, the method of poisoned substrates was used, in which macerates of plants in a concentration of 2% and 5% were added to the glucose-potato substrate (PDA). The experiment was carried out for two weeks in duplicate. The growth of mycelium and the ability to form spores were assessed. The research shows that plant additives have a different degree of impact on the studied phytopathogens. The most effective turned out to be cinnamon, which completely inhibited the growth of all the fungi examined. The presence of the antagonistic fungus *Trichoderma viride* has definitely influenced the development of the studied fungi. In all cases, the rapid growth of *T. viride* mycelium was noted and inhibition of phytopathogenic fungal growth.

Introduction

In modern agriculture, according to the integrated plant protection in force since 2014, in the fight against phytopathogens, biological methods of plant protection are becoming increasingly important. These methods use natural antagonistic mechanisms between microorganisms: antibio-

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sis, competition and parasitism, as well as phytoncides present in plants. Therefore, research on alternative and effective plant protection agents focuses on the search for antagonistic microorganisms secreting antibiotic substances, volatile compounds and lytic enzymes, as well as plant resistance inducing agents (SANTOS and MARQUINA 2004, EL-TARABILYA and SIVASITHAMPARAMB 2006, MATYJASZCZYK and SOBCZAK 2011, WALKOWIAK and KRZYŚKO-ŁUPICKA 2014), thus inhibiting the growth of pathogenic microorganisms. Important biological agents with antibiotic, competitiveness and parasitic abilities include Trichoderma (KASHYAP et al. 2017). Numerous studies prove different efficacy of these fungi (*T. harzianum* Rifai*, T. koningii* Oudem.*, T. viride* Pers.) in relation to numerous phytopathogens (WOJTKOWIAK-GĘBAROWSKA 2006). Therefore, biopreparations are available on the market based on *Trichoderma* spp. (BRIAN and HEMMING 2008), which can be successfully used, among others in greenhouses, in crops under covers, conducted using conventional methods as well as in organic crops. Recent studies show on this subject is also focused on substances of plant origin (KRĘCIDŁO and KRZYŚKO-ŁUPICKA 2017). The compounds contained in various species of plants – phytoncides, have been known for a long time as a natural passive chemical defense against phytopathogens (KOZŁOWSKA 2007). Numerous of them with fungistatic properties have been used to develop biological agents, such as Biosept 33 based on grapefruit seed extract or Bioczos based on garlic (SZOPIŃSKA et. al. 2007, MARJANSKA-CICHON and SAPIEHA-WASZKIEWICZ 2010, ZYDLIK 2008). However, new, effective plant compounds are still being sought for acting against numerous dangerous pathogenic fungi.

The aim of the work was to determine the effect of three plant additives with different concentrations, as well as the influence of the *Trichoderma viride* saprotrophic fungus on the growth and development of several species of phytopathogenic fungi, economically important.

Material and Methods

Eight fungal species were selected for the study: *Botrytis cinerea* (Pers.)*, Fusarium avenaceum* (Fr.) Sacc.*, F. culmorum* (W.G. Sm.) Sacc.*, F. oxysporum* (Schltdl)*, F. solani* (Mart.) Sacc.*, Monilinia fructigena* (Honey)*, Sclerotinia sclerotiorum* (Lib.) de Bary and *Trichothecium roseum* (Pers.) Link. The strains were isolated from diseased crop plants (pumpkin, apple, carrot, wheat, vines). For the isolation of fungi the method of moist chambers was used, to which sick parts of plants were laid out. After 24h, in order to obtain pure cultures, fungal isolates were passaged onto

standard PDA medium used in phytopathological diagnostics (MARCINKOWSKA 2003). In order to carry out the experiment, cultures were carried out in petri dishes using the poisoned substrate method. The dry mass of three plant species: mint (*Mentha*), cinnamon (*Cinnamomum*) and English herb (*Pimenta dioica*), were added to 15 ml of liquid PDA medium in two variants:

– 2% concentration: the substrate PDA prepared in 3 flasks was successively added mint, cinnamon, allspice (2 g of dry matter/100 ml of medium);

– 5% concentration: the substrate PDA prepared in 3 flasks was successively added mint, cinnamon, allspice (5 g of dry matter/100 ml of medium).

The experiment was carried out in a few repetitions and in 2 variants.

Dry mass were obtained by grinding the dried parts of plants in a sterile mortar until fine dust was obtained. The control sample consisted of isolates of the tested fungal species sown on a PDA medium without herbal additives. In order to determine the effect of saprotrophic *Trichoderma viride*, the tested species of phytopathogenic fungus and *T. viride* were placed next to each other on Petri dishes with PDA substrate. The Petri dish with isolates was sealed with parafilm. The experiment was carried out for two weeks, in duplicate, at room temperature of 22°C. During daily observations, the diameter of the mycelium was measured and the time of spore formation was recorded. For this purpose, preparations from Gerlach's culture were made. Under sterile conditions, pieces of adhesive tape with a length of about 3 cm were prepared and imprints of grown mushroom colonies were made. This material was transferred to the primary slide, with the adhesive side up, a drop of blue with lactofenol applied and covered with a coverslip. The preparations were viewed under an OLYMPUS Bx4 optical microscope.

On the basis of the obtained results, the percentage of mycelial growth inhibition was calculated according to the formula:

$$\text{pzw} = \frac{K_0 - F}{K_0} \cdot 100$$

where :
pzw – percent inhibition of growth;
K_0 – diameter of the culture in the control combination (culture covering the whole pan);
F – diameter in combination with plant additive.

The given values were averaged.

Results and Discussions

Different effects of plant additives on the development of the fungi studied were found. In all cases, total inhibition of mycelial growth was noted by the addition of cinnamon at a concentration of 2% and 5% (Table 1).

Table 1

Percentage of growth inhibition (pzw) of the fungi examined on the substrate with plant additives

Herbal additives / Species of fungi	Cinnamon		English herb		Mint	
	2%	5%	2%	5%	2%	5%
B. cinerea	100%	100%	55%	100%	77%	100%
F. avenaceum	100%	100%	68%	100%	2%	55%
F. culmorum	100%	100%	66%	100%	44%	10%
F. oxysporum	100%	100%	44%	81%	1%	50%
F. solani	100%	100%	31%	81%	22%	44%
M. fructigena	100%	100%	66%	100%	83%	100%
S. sclerotiorum	100%	100%	77%	66%	44%	55%
T. roseum	100%	100%	44%	77%	33%	66%

Available literature indicates that the ground cinnamon bark is fungistatic and bactericidal. It results from the oil substances contained in it – mainly cinnamon oil (ZOHREH et. al. 2011, PIEKUTOWSKA 2017), which includes cinnamaldehyde. Research conducted in 2005, by JHAM et. al., showed that cinnamaldehyde is the main fungicide of cinnamon bark, while other components addively or synergistically affect the total fungisticity of cinnamon. Similar results were obtained by other scientists testing the effectiveness of cinnamon bark extracts in inhibiting the growth of fungi of the genus *Fusarium*. The largest inhibitory effect, *in vitro* and *in vivo*, using cinnamon was recorded by EL-MOUGY and ABDEL-KADER (2007).

The addition of English herb (5%) to four fungal species was also successful: *Botrytis cinerea, Fusarium avenaceum, Fusarium culmorum* and *Monilinia fructigena*. The most important phytoncide contained in fruits of English herb is essential oil, which consists of: eugenol (60–80%), methyl-eugenol (20–30%), feldenne, cineole, cariophylene and palmitic acid known for its antimicrobial and antifungal properties (CZERWIŃSKA and PIOTROWSKI 2005, BERTHOLD-PLUTA and KURZYŃSKA 2010).

The weakest fungistatic properties were observed in the case of mint, the addition of which (5%) inhibited the growth of only *B. cinerea* and *M. fructigena* (Figure 1, Figure 2). Mint leaves contain about 3% essential oil, tannins, phenolic acids, triterpenes, carotenoids and flavonoids.

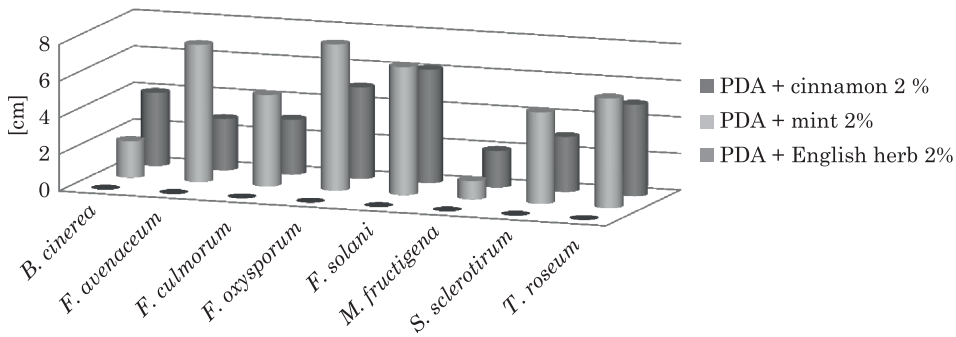

Fig. 1 Effect of vegetable additives (2%) on PDA substrate for mycelium development

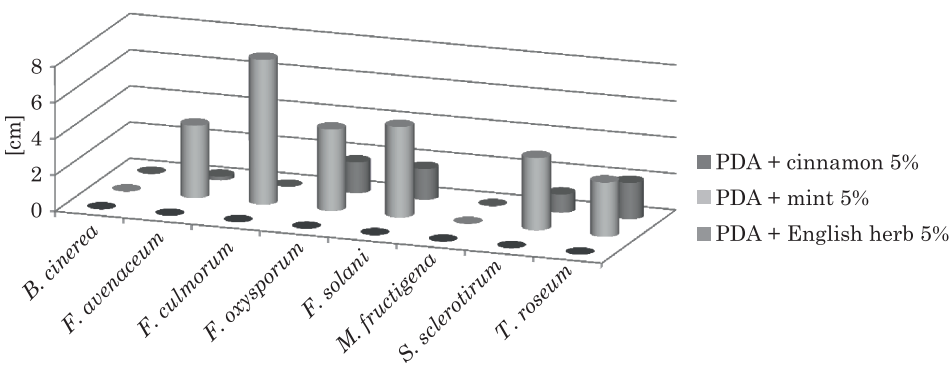

Fig. 2. Effect of vegetable additives (5%) on PDA substrate for mycelium development

Contained in the essential oil – menthol, has strong antimicrobial properties. Some authors claim that this oil affects the growth of mycelia of yeast-like and mold fungi (KUSIAK et. al. 2010), and also inhibits the production of mycotoxins, e.g. ochratoxin A by *Aspergillus parasiticus* (FERDES and UNGUREANU 2012). Studies carried out by FERDES and UNGUREANU (2012) showed a strong effect of peppermint oil on inhibiting the growth of *F. oxysporum* mycelium, which was not observed in our own studies. This is probably due to the fact that FERDES and UNGUREANU (2012) used a pure, extracted peppermint oil in which the concentration of active compounds was very high, which had an effect on inhibiting the growth of the *Fusarium* fungi tested. However, the higher concentration

of dried mint leaves added to the medium increased the degree of mycelial growth inhibition of the phytopathogens tested. EJECHI et. al. (1997) also drew a similar conclusion, stating the effectiveness of biopreparations with increasing their concentration.

The antagonistic effect of *Trichoderma viride* on the development of the fungi studied was found. The largest was demonstrated in the case of *F. solani*, whose diameter of the mycelium was only 1 cm (pzw = 88%). In the remaining species, the percentage of mycelial growth inhibition ranged from 66% to 82%. The exception was *F. culmorum* mushroom, which in the presence of *T. viride* developed abundant mycelium (pzw = 44%) – Figure 3.

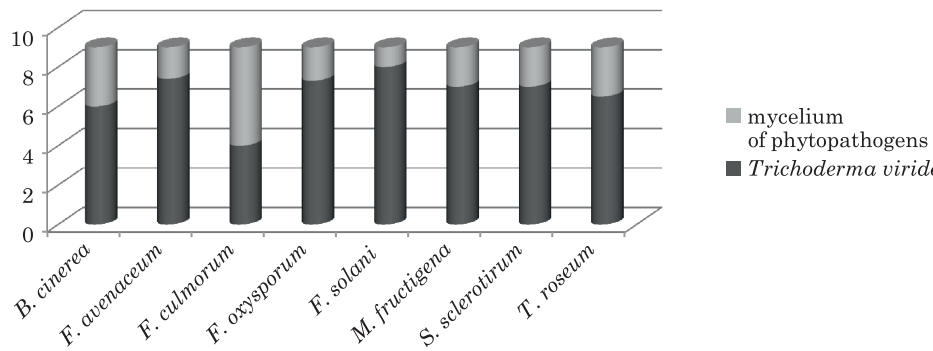

Fig. 3. Effect of *Trichoderma viride* on PDA substrate for fungal growth

Trichoderma viride is a rhizosphere species. Its antagonistic effect on pathogens results from aggressive competition for nutrients and space, as well as from the production of various active substances that inhibit the growth of other pathogens. These substances include lytic enzymes that interact with antibiotics (WOJTKOWIAK-GĘBAROWSKA 2006).

An inhibition of spore growth was observed in 3 examined fungal species. The addition of 5% English herb delayed the formation of *Fusarium solani* spores by 6 days and at *F. oxysporum* by 3 days compared to the control. In the case of the addition of 5% mint, the delay in spore formation was noted in *M. fructigena*, whereas in *F. avenaceum* no fungal spores were observed in the presence of this plant supplement. In the remaining species studied, forming conidial spores, their formation was not inhibited compared to the control.

Conclusions

Summing up, the results obtained in the conducted study indicate the different properties of fungistatic compounds contained in plants. The most effective was the addition of cinnamon, which already at a concentration of 2% completely inhibited the growth of mycelium of the tested isolates. Further research should be directed to the use of this herbal supplement as an available biological agent in the protection of plants against phytopathogens. *T. viride* significantly reduced the growth of mycelium of all tested fungi, with the exception of *F. culmorum*.

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