PROGRESS IN PLANT PROTECTION
64 (2): 75–88, 2024
eISSN 2084-4883

DOI: 10.14199/ppp-2024-008
Published online: 17.05.2024

Received: 05.03.2024 / Accepted: 18.04.2024

ARTYKUŁ ORYGINALNY

Ganoderma lucidum i *Pleurotus ostreatus* w testach na antagonistyczne oddziaływanie wobec patogenicznych grzybów i bakterii dla roślin pomidora

Ganoderma lucidum and *Pleurotus ostreatus* in tests for antagonistic effects against fungal and bacterial pathogens for tomato plants

Weronika Zenelt*, Katarzyna Sadowska, Sylwia Stępniewska-Jarosz, Natalia Łukaszewska-Skrzypniak, Natasza Borodynko-Filas

Streszczenie

Grzyby wielkoowocnikowe, takie jak *Ganoderma lucidum* i *Pleurotus ostreatus*, znane są z właściwości prozdrowotnych i wartości kulinarnej. Badano ich zdolność do hamowania wzrostu patogenicznych grzybów i bakterii roślin pomidora. *Ganoderma lucidum* i *P. ostreatus* efektywnie hamowały wzrost *Alternaria solani* i *Botrytis cinerea*. Obserwowano również ograniczone hamowanie wzrostu bakterii, z największym efektem dla *Clavibacter michiganensis* subsp. *michiganensis* pod wpływem *G. lucidum* oraz dla *Pseudomonas syringae* pod wpływem *P. ostreatus*. To pierwsze doniesienie o antagonistycznym działaniu *P. ostreatus* na patogeny bakteryjne roślin pomidora. Przeprowadzone badania mogą przyczynić się do opracowania ekologicznych strategii kontroli zakażeń roślin pomidora, minimalizując ich negatywne skutki dla środowiska i zdrowia ludzkiego.

Słowa kluczowe: grzyby patogeniczne, bakterie patogeniczne, choroby pomidora, działanie antybakteryjne, kontrola biologiczna, grzyby wielkoowocnikowe

Abstract

Large fruiting body fungi, such as *Ganoderma lucidum* and *Pleurotus ostreatus*, are known for their health benefits and culinary value. Their ability to inhibit the growth of pathogenic fungi and bacteria in tomato plants has been investigated. Both *G. lucidum* and *P. ostreatus* effectively inhibited the growth of *Alternaria solani* and *Botrytis cinerea*. Limited inhibition of bacterial growth was also observed, with the greatest effect on *Clavibacter michiganensis* subsp. *michiganensis* under the influence of *G. lucidum* and on *Pseudomonas syringae* under *P. ostreatus*. This is the first report of *P. ostreatus* antagonistic action against bacterial pathogens of tomato plants. Our research may contribute develop to ecological strategies for controlling tomato plant infections, minimizing their negative environmental and human health effects.

Key words: phytopathogenic fungi, phytopathogenic bacteria, tomato diseases, antimicrobial activity, biological control, macrofungi

Instytut Ochrony Roślin – Państwowy Instytut Badawczy
ul. Władysława Węgorka 20, 60-318 Poznań
*corresponding author: w.zenelt@iorpib.poznan.pl

Wstęp / Introduction

Macroscopic fungi, commonly referred to as macromycetes, constitute a diverse and artificial group of fungi that give rise to large fruiting bodies visible to the naked eye. These mushrooms exhibit a variety of shapes, characterized by a rich array of colors, smells, and flavors. In contrast, microscopic fungi (micromycetes) primarily serve as subjects of microbiological and phytopathological research (Moradali et al. 2007). Currently, approximately 10,000 mushroom species are known, with around 300 recognized as medicinal due to their beneficial properties (Rezghi Jahromi and Mozafary 2021). Some of these mushrooms have been utilized in traditional therapies since as early as 3000 BC, particularly in China, Japan, Korea, and among Slavic peoples (Moradali et al. 2007; Rezghi Jahromi and Mozafary 2021). Numerous studies support the production of biologically active substances by these fungi (De Silva et al. 2012; Krupodorova et al. 2016; Pala et al. 2019; Sharma et al. 2019; Seweryn et al. 2021; Mustafin et al. 2022; Saludares et al. 2023). They serve as a reservoir of valuable nutrients, including amino acids, glycopeptides, carbohydrates, amino sugars, sugar acids, polysaccharides, glycophene, ß-glucan, vitamins, unsaturated fatty acids, and ascorbic acid. Additionally, they are rich sources of minerals such as potassium, phosphorus, sodium, calcium, magnesium, copper, zinc, and iron (Atri et al. 2013; Younis et al. 2015; Bhardwaj et al. 2020; Raman et al. 2021; Lesa et al. 2022). While these fungi hold potential for health-promoting applications, there is a growing interest in exploring their use for plant protection against pathogens. The escalating concern surrounding chemical plant protection products, including fungicides and antibiotics, and their adverse impact on the environment and human health underscores the need for alternative strategies to combat crop pathogens.

Tomato (*Solanum lycopersicum* L.) stands as one of the most cultivated vegetables globally. According to FAOSTAT, the global production in 2021 reached approximately 189,133,955 tons (FAOSTAT 2021). Despite its prominence, tomato cultivation is often susceptible to diseases caused by fungal and bacterial pathogens. Notably, phytopathogenic fungi such as *Alternaria solani* (Chaerani and Voorrips 2006; Fritz et al. 2006; Kumar et al. 2013; Attia et al. 2020), *Botrytis cinerea* (Williamson et al. 2007; Ge et al. 2015; Jin and Wu 2015; Wakeham et al. 2016) and *Fusarium oxysporum* (Takken and Rep 2010; McGovern 2015; Srinivas et al. 2019) pose significant threats. Additionally, we focused on one of the most detrimental bacteria in tomato crops *Clavibacter michiganensis* subsp. *michiganensis* (Gartemann et al. 2003; Mansfield et al. 2012; Valenzuela et al. 2018), as well as less harmful *Pseudomonas syringae* (Kozik and Sobiczewski 2000; Buell et al. 2003; Zhao et al. 2003) and the lesser-known *Pseudomonas viridilivida* (Zenelt et al. 2021).

Ganoderma lucidum and *Pleurotus ostreatus*, the species selected for this study, are well-established members of the macro fungi category. *Ganoderma lucidum*, commonly known as *reishi*, is renowned for its diverse array of bioactive chemical compounds. Numerous studies indicate that *G. lucidum* extracts exhibit antimicrobial activity, influencing the growth of pathogenic microorganisms, both for plants (Shahid et al. 2016; da Cruz et al. 2019; Robles-Hernández et al. 2021) and for animals and humans (Grys et al. 2011; Kamble et al. 2011; Rezghi Jahromi and Mozafary 2021; Mustafin et al. 2022). *Pleurotus ostreatus*, also recognized as the *oyster mushroom*, is a popular edible species with ongoing research into its antifungal and antibacterial properties (Kumar and Yadav 2014; Owaid et al. 2015; Younis et al. 2015; Parola et al. 2017; El Domany et al. 2018; Gashaw and Getu 2021; Ianni et al. 2021). However, there is limited information regarding their activity against bacterial pathogens affecting plants. Most researchers have primarily focused on bacteria pathogenic to humans (Vamanu 2013; Al-Bahrani et al. 2017; Gashaw et al. 2020; Waktola and Temesgen 2020; Lesa et al. 2022).

The objective of this study was to explore the antagonistic impact of selected macrofungi, *G. lucidum* and *P. ostreatus*, on well-known and prevalent pathogenic fungi in tomato crops: *A. solani*, *B. cinerea*, and *F. oxysporum*. Additionally, we investigated the influence of these macrofungi on pathogenic bacteria, namely *C. michiganensis* subsp. *michiganensis*, *P. syringae*, and *P. viridilivida*, commonly found in tomato crops. To our knowledge, this is the first test for the antagonistic effect of *P. ostreatus* mycelium on tomato pathogenic bacteria.

Materiały i metody / Materials and methods

Izolaty grzybów wielkoowocnikowych / Macrofungi samples

The strains of two macrofungi were obtained from the collection of the Bank of Plant Pathogen of the Institute of Plant Protection – National Research Institute in Poznań (Poland). The following fungal strains were used: *G. lucidum* (accession no. 2480) and *P. ostreatus* (accession no. 2481). Mushrooms were grown on potato dextrose agar (PDA Difco™, Becton Dickinson and Company) for 7 days at 24°C until mycelium grew over the entire surface of the Petri dish.

Izolaty grzybów patogenicznych / Pathogenic fungal samples

Three strains of tomato plant pathogenic fungi were obtained from the collection of the Bank of Plant Pathogen of the Institute of Plant Protection – National Research Institute in Poznań (Poland). The following fungal strains were used: *A. solani* (accession no. 2046), *B. cinerea* (accession no. 2235) and *F. oxysporum* (accession no. 2029). The fungi were grown on potato dextrose agar (PDA Difco™, Becton Dickinson and Company) for 7 days at 24°C until spore formation.

Izolaty bakterii patogenicznych / Pathogenic bacteria samples

Three strains of tomato plant pathogenic bacteria were obtained from the collection of the Bank of Plant Pathogen of the Institute of Plant Protection – National Research Institute in Poznań (Poland). The following bacterial strains were used: *C. michiganensis* subsp. *michiganensis* (accession no. 2457), *P. syringae* (accession no. 2146) and *P. viridilivida* (accession no. 2484). Bacteria were grown on soy agar (TSA, Sigma Aldrich Company Ltd.) and incubated for 24 hours at 24°C.

Identyfikacja izolatów grzybowych i bakteryjnych / Fungi and bacteria samples identification

Selected isolates of macrofungi and pathogenic fungi were identified molecularly and morphologically. For molecular identification a pair of primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') were used (Bertini et al. 1999; Vancov and Keen 2009). DNA was isolated from pure fungal strains using a kit from Norgen Biotek Corp. (Ontario, Canada) according to manufacturer's instructions. Two microliters of product was the template in the PCR performed using GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA). The reaction profile was: 5 min – 94°C, 32 cycles of 15 s – 94°C, 15 s – 56°C and 40 s – 72°C, with final elongation 7 min – 72°C. The appropriate size of the obtained PCR products was verified on 1% agarose gel and sequenced by an outsourced company (Genomed S.A., Warsaw, Poland). Obtained nucleotide sequences were aligned using BioEdit software (v. 7.2) (Hall 1999) to obtain consensus sequences. Each consensus sequence was made out of three sequencing reads. Next, the consensus sequences were identified using BLAST tool (https://blast.ncbi.nlm.nih.gov/), and deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/). The morphological and microscopic characterization of selected isolates was carried out based on the identify fungi guide.

Selected bacterial isolates were identified biochemically, using the BIOLOG® Gen III system (v. 2.8 database) (BIOLOG®, Hayward, USA) according to manufacturer's instructions.

Interakcja grzybni grzybów wielkoowocnikowych z grzybami patogenicznymi / Macrofungi mycelium and pathogenic fungi interaction

The antagonistic effect of selected macrofungi against selected pathogenic fungi to tomato was assessed on the basis of the dual culture test (Asif et al. 2022). Agar discs of 6 mm diameter were cut from the edge of the clean PDA medium (1 cm from the edge of the plate), and replaced with a disc from the previously cultivated pathogenic fungus. At the same time, a culture disc of a macrofungus of 6 mm diameter was placed in the center of the dish. The dishes were incubated at 28°C for 8 days. The antagonistic effect was measured after 4, 6 and 8 days of incubation based on the growth rate of the pathogenic fungus colony [mm] towards the macrofungus (T) and based on the growth radius of the pathogenic fungus colony on the control plate (C).

Interakcja grzybni grzybów wielkoowocnikowych z bakteriami patogenicznymi / Macrofungi mycelium and pathogenic bacteria interaction

The antagonistic effect of selected macrofungi against selected pathogenic bacteria to tomato was assessed on the basis of the Weller's method (Weller et al. 1985). Two 6 mm diameter agar discs were cut opposite each other in a petri dish with sterile PDA medium. A sterile disc was collected from a 7-day culture of the macrofungus and then placed in one of the wells, next plates were incubated for 48 hours at 24°C. After 48 hours, a disk of pathogenic bacteria was collected from the 24-hour culture and then placed in the second well opposite the previously inoculated macrofungus. The dish was incubated at 24 degrees (Ofodile et al. 2011). The antagonistic effect was measured after 3, 5 and 7 days of incubation based on the growth rate of the pathogenic bacterial colony [mm] in the dual culture (T) and based on the growth radius of the pathogenic bacterial colony on the control plate (C).

The macrofungus – pathogenic fungus combination was measured one day later due to the longer growth of the fungi compared to the bacteria.

Procentowe zahamowanie rozrostu wybranych patogenicznych grzybów i bakterii / Percentage inhibition of radial growth of chosen pathogenic fungi and bacteria

The results were converted into percentage inhibition of colony growth of both pathogenic fungi and bacteria. PIRG (percentage inhibition of radial growth) (Owaid et al. 2015; Sonawane et al. 2015) was calculated from the formula:

$$\text{PIRG (\%)} = \frac{C-T}{C} \times 100,$$

where:

C – control colony growth diameter (pathogenic fungus / pathogenic bacteria),

T – colony growth diameter of pathogenic fungus / bacteria in dual culture.

The average value of the measurements was taken for the calculations.

Analiza statystyczna / Statistical analysis

The variables in each group were described by the arithmetic mean ± standard deviation. The groups were compared using ANOVA for independent variables. The post-hoc analysis was performed. To indicate significant differences of data the LSD Tukey test (least significant difference) was performed. The significance level was 0.05. Differences at $p < 0.05$ were considered to be significant. Five replicates

were used in the experiment for each combination. The calculations were carried out in the Statistica 12.0 program.

Wyniki i dyskusja / Results and discussion

All fungal isolates used in the experiment were molecularly identified based on the ITS region. Bacterial isolates were biochemically identified using the BIOLOG system. Table 1 presents a list of all isolates.

The average values of inhibitory properties of macrofungi against pathogenic organisms for tomato plants are shown in table 2 for pathogenic fungi, and in table 3 for pathogenic bacteria.

The growth of the pathogenic fungi *A. solani* and *F. oxysporum* was notably suppressed by both macrofungi, *G. lucidum* and *P. ostreatus*, throughout the entire incubation period. In the case of the pathogenic fungus *B. cinerea*, incubation with *G. lucidum* led to substantial initial inhibition, but this effect diminished significantly over time, reaching 0% inhibition by the end of the incubation period. On the other hand, *P. ostreatus* initially inhibited the growth of *B. cinerea*, but after 6 days, a noteworthy expansion of *B. cinerea* mycelium was observed. Eventually, the presence of *P. ostreatus* mycelium significantly curtailed the growth of the pathogenic fungus (tab. 4, photo 1–3, fig. 1, 2).

The growth of the pathogenic bacteria *C. michiganensis* subsp. *michiganensis* was significantly and consistently inhibited over time in the presence of the macrofungus *G. lucidum*. By the end of the incubation period, the Percentage Inhibition of Radial Growth (PIRG) for this combination recorded the highest value among all bacterial-

Tabela 1. Identyfikacja grzybów wielkoowocnikowych i grzybów patogenicznych na podstawie regionu ITS; identyfikacja biochemiczna bakterii patogenicznych z wykorzystaniem systemu BIOLOG®
Table 1. Macrofungi and pathogenic fungi identification based on the ITS region; pathogenic bacteria identification based on biochemically identification using the BIOLOG® system

		Numer akcesyjny GenBank GenBank given accession number	Wartość Value Query cover	Procent zbieżności Percent identity	Numer akcesyjny Accession number
Grzyby wielkowocnikowe Macrofungi	*Ganoderma lucidum*	OQ947057	99%	97.00%	MG911000.1
	Pleurotus ostreatus	OQ947058	100%	98.17%	LN849458.1
Grzyby patogeniczne Pathogenic fungi	*Alternaria solani*	OQ947054	100%	99.82%	MN121432.1
	Botrytis cinerea	OQ947055	100%	100%	MN489448.1
	Fusarium oxysporum	OQ947056	100%	99.81%	MZ724816.1
		Identyfikacja BIOLOG® BIOLOG® identification	Podobieństwo Similarity	Rozstęp Distance	
Bakterie patogeniczne Pathogenic bacteria	*Pseudomonas syringae*	*Pseudomonas syringae*	0.664	4.586	–
	Pseudomonas viridilivida	*Pseudomonas viridilivida*	0.785	4.398	–
	Clavibacter michiganensis subsp. *michiganensis*	*Clavibacter michiganensis*	0.894	4.699	–

Tabela 2. Średnia wartość hamowania wzrostu grzybów patogenicznych pod wpływem grzybni grzybów wielkoowocnikowych w teście podwójnych kultur [mm]
Table 2. The average value of growth inhibition of pathogenic fungi under the influence of macrofungus mycelium in dual culture test [mm]

	Średnia ± odchylenie standardowe [mm] Mean ± standard deviation [mm]		
I pomiar (4. dzień inkubacji) I measurement (4th day of incubation)	*Alternaria solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Ganoderma lucidum	14.4 ± 0.89	25.4 ± 1.51	12.0 ± 1.0
p-value*	0.000889	0.000140	0.309147
Pleurotus ostreatus	17.0 ± 1.22	27.0 ± 0.7	14.0 ± 0.7
p-value	0.421269	0.000140	0.999977
Kontrola (wzrost grzybów patogenicznych bez obecności grzybów wielkoowocnikowych) Control (growth of pathogenic fungi without macrofungi)	19.2 ± 0.83	50.8 ± 3.76	14.4 ± 0.89

Tabela 2. Średnia wartość hamowania wzrostu grzybów patogenicznych pod wpływem grzybni grzybów wielkoowocnikowych w teście podwójnych kultur [mm] – cd.
Table 2. The average value of growth inhibition of pathogenic fungi under the influence of macrofungus mycelium in dual culture test [mm] – continued

	Średnia ± odchylenie standardowe [mm] Mean ± standard deviation [mm]		
II pomiar (6. dzień inkubacji) II measurement (6th day of incubation)	*Alternaria solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Ganoderma lucidum	15.0 ± 0.7	65.0 ± 5.87	12.0 ± 1.0
p-value	0.000140	0.000149	0.000149
Pleurotus ostreatus	18.2 ± 0.83	66.0 ± 4.0	14.0 ± 0.7
p-value	0.000162	0.000216	0.000686
Kontrola (wzrost grzybów patogenicznych bez obecności grzybów wielkoowocnikowych) Control (growth of pathogenic fungi without macrofungi)	27.8 ± 1.48	75.0 ± 0.0	22.0 ± 2.0
III pomiar (8. dzień inkubacji) III measurement (8th day of incubation)	*Alternaria solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Ganoderma lucidum	15.0 ± 1.0	75.0 ± 0.0	12.0 ± 1.0
p-value	0.000140	1.000000	0.000140
Pleurotus ostreatus	18.4 ± 0.89	27.0 ± 2.44	14.0 ± 0.7
p-value	0.000140	0.000140	0.129193
Kontrola (wzrost grzybów patogenicznych bez obecności grzybów wielkoowocnikowych) Control (growth of pathogenic fungi without macrofungi)	36.6 ± 2.07	75.0 ± 0.0	29.6 ± 2.19

*p-value < 0,05 oznacza wartości istotne statystycznie – p-value < 0.05 indicate statistically significant values

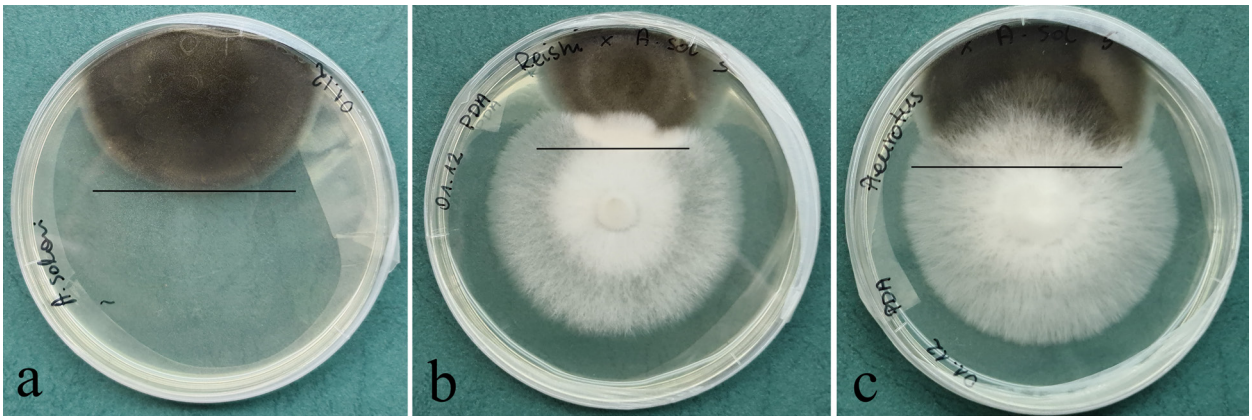
Tabela 3. Średnia wartość hamowania wzrostu bakterii patogenicznych pod wpływem grzybni grzybów wielkoowocnikowych w metodzie Wellera [mm]
Table 3. The average value of growth inhibition of pathogenic bacteria under the influence of macrofungus mycelium in Weller's method [mm]

I pomiar (3. dzień inkubacji) I measurement (3th day of incubation)	Średnia ± odchylenie standardowe [mm] Mean ± standard deviation [mm]		
	Pseudomonas viridilivida	*Pseudomonas syringae*	*Clavibacter michiganensis* subsp. *michiganensis*
Ganoderma lucidum	12.2 ± 1.92	7.4 ± 0.54	9.6 ± 0.89
p-value*	0.995931	0.627234	0.627234
Pleurotus ostreatus	12.4 ± 1.94	7.0 ± 1.22	9.6 ± 0.89
p-value	0.973949	0.302093	0.627234
Kontrola (wzrost bakterii patogenicznych bez obecności grzybów wielkoowocnikowych) Control (growth of pathogenic bacteria without macrofungi)	11.6 ± 0.54	8.8 ± 0.83	11.0 ± 0.7
II pomiar (5. dzień inkubacji) II measurement (5th day of incubation)	*Pseudomonas viridilivida*	*Pseudomonas syringae*	*Clavibacter michiganensis* subsp. *michiganensis*
Ganoderma lucidum	15.4 ± 0.54	7.3 ± 0.44	10.6 ± 0.89
p-value	0.902567	0.009920	0.00149
Pleurotus ostreatus	16.0 ± 1.0	7.4 ± 0.54	12.4 ± 1.34
p-value	0.318184	0.015616	0.083393
Kontrola (wzrost bakterii patogenicznych bez obecności grzybów wielkoowocnikowych) Control (growth of pathogenic bacteria without macrofungi)	14.6 ± 0.89	9.6 ± 0.89	14.2 ± 1.3
III pomiar (7. dzień inkubacji) III measurement (7th day of incubation)	*Pseudomonas viridilivida*	*Pseudomonas syringae*	*Clavibacter michiganensis* subsp. *michiganensis*
Ganoderma lucidum	16.0 ± 1.22	7.8 ± 0.83	10.6 ± 0.89
p-value	0.333112	0.108791	0.000140
Pleurotus ostreatus	16.0 ± 0.7	7.4 ± 0.54	13.6 ± 1.34
p-value	0.333112	0.027070	0.012575
Kontrola (wzrost bakterii patogenicznych bez obecności grzybów wielkoowocnikowych) Control (growth of pathogenic bacteria without macrofungi)	17.6 ± 1.51	9.8 ± 0.83	16.2 ± 1.3

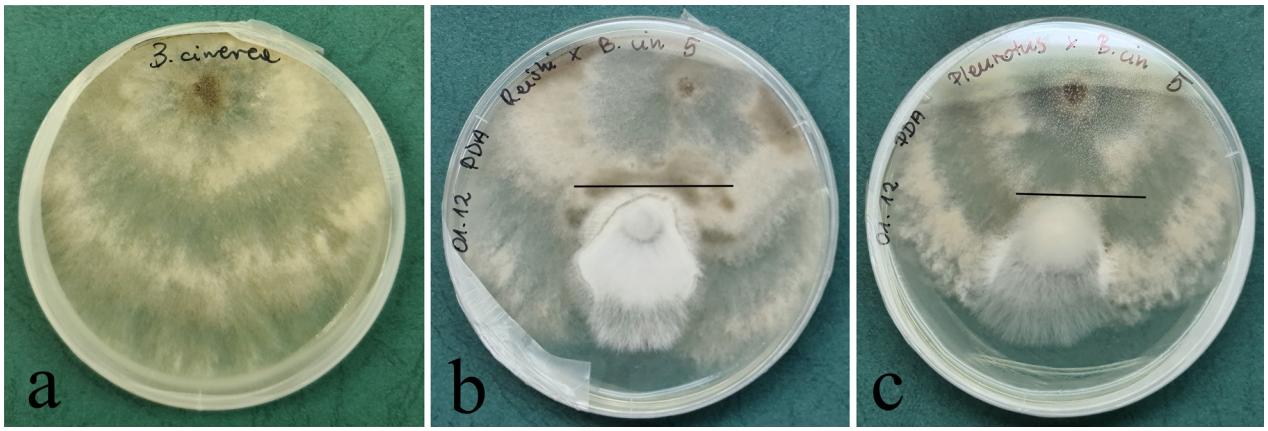
*p-value < 0,05 oznacza wartości istotne statystycznie – p-value < 0.05 indicate statistically significant values

Tabela 4. Procentowe zahamowanie rozrostu wybranych grzybów patogenicznych w ciągu kolejnych dni pomiaru w obecności grzybni grzybów wielkoowocnikowych (PIRG)
Table 4. Percentage inhibition of radial growth of selected pathogenic fungi during the following measurement days in the presence of macrofungi (PIRG)

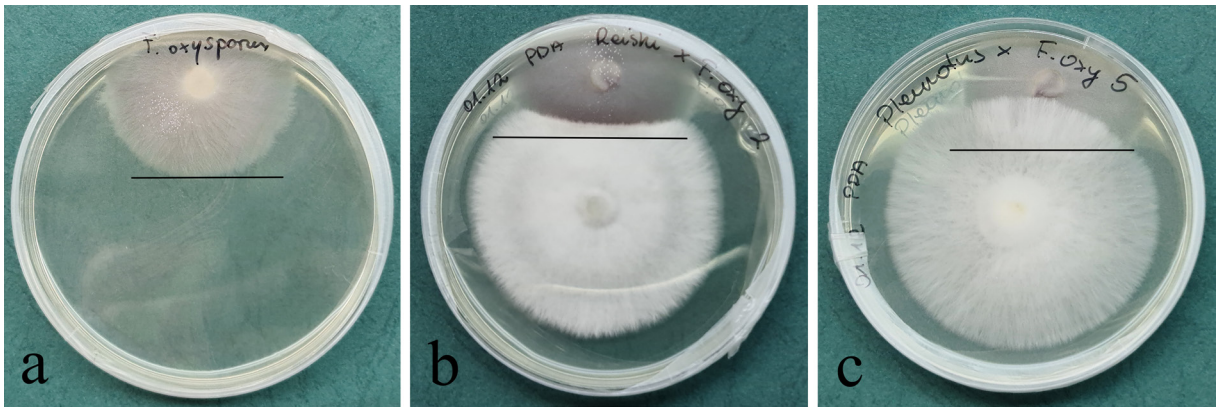
I pomiar (4. dzień inkubacji) I measurement (4th day of incubation)	*Alternaria solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Ganoderma lucidum *Pleurotus ostreatus*	25% 11.45%	50% 46.85%	16.6% 2.77%
II pomiar (6. dzień inkubacji) II measurement (6th day of incubation)	*Alternaria solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Ganoderma lucidum *Pleurotus ostreatus*	46% 34.53%	13.33% 12%	45% 36.36%
III pomiar (8. dzień inkubacji) III measurement (8th day of incubation)	*Alternaria solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Ganoderma lucidum *Pleurotus ostreatus*	59.01% 49.72%	0% 64%	59.45% 52.7%



Fot. 1. Test podwójnych kultur: (a) wzrost kontrolny grzybni *Alternaria solani*, (b) wzrost grzybni *Alternaria solani* w obecności *Ganoderma lucidum*, (c) wzrost grzybni *Alternaria solani* w obecności *Pleurotus ostreatus*; pomiar po 8 dniach
Photo 1. Dual culture test: (a) control growth of *Alternaria solani* mycelium, (b) growth of *Alternaria solani* mycelium in the presence of *Ganoderma lucidum*, (c) growth of *Alternaria solani* mycelium in the presence of *Pleurotus ostreatus*; measurement after 8 days

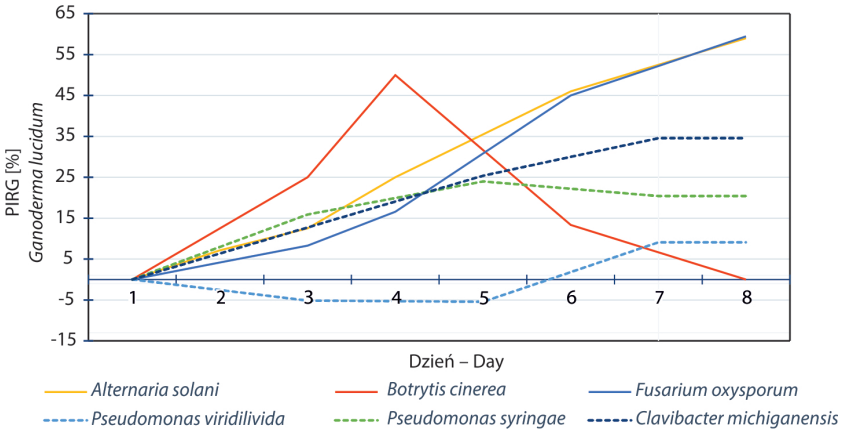


Fot. 2. Test podwójnych kultur: (a) wzrost kontrolny grzybni *Botrytis cinerea*, (b) wzrost grzybni *Botrytis cinerea* w obecności *Ganoderma lucidum*, (c) wzrost grzybni *Botrytis cinerea* w obecności *Pleurotus ostreatus*; pomiar po 8 dniach
Photo 2. Dual culture test: (a) control growth of *Botrytis cinerea* mycelium, (b) growth of *Botrytis cinerea* mycelium in the presence of *Ganoderma lucidum*, (c) growth of *Botrytis cinerea* mycelium in the presence of *Pleurotus ostreatus*; measurement after 8 days



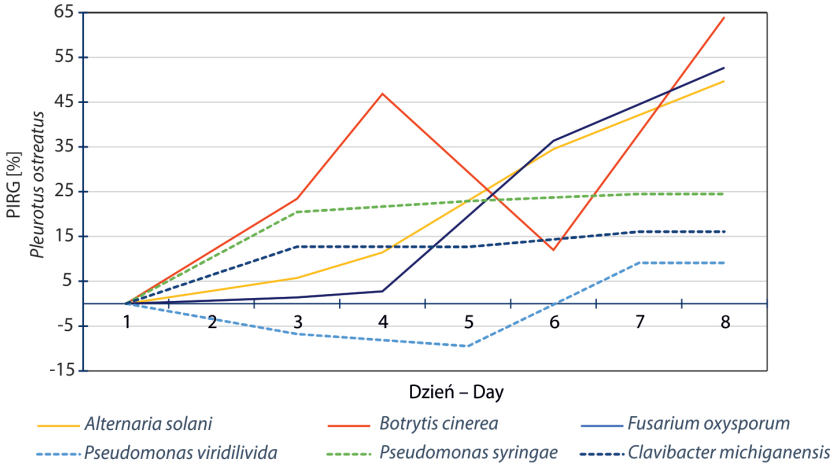
Fot. 3. Test podwójnych kultur: (a) wzrost kontrolny grzybni *Fusarium oxysporum*, (b) wzrost grzybni *Fusarium oxysporum* w obecności *Ganoderma lucidum*, (c) wzrost grzybni *Fusarium oxysporum* w obecności *Pleurotus ostreatus*; pomiar po 8 dniach

Photo 3. Dual culture test: (a) control growth of *Fusarium oxysporum* mycelium, (b) growth of *Fusarium oxysporum* mycelium in the presence of *Ganoderma lucidum*, (c) growth of *Fusarium oxysporum* mycelium in the presence of *Pleurotus ostreatus*; measurement after 8 days



Rys. 1. Zmiana wartości PIRG w czasie dla wybranych patogenicznych grzybów i bakterii dla roślin pomidora; wpływ grzyba wielkowocznikowego *Ganoderma lucidum*

Fig. 1. Change in PIRG value over time for selected fungal and bacterial pathogens for tomato plants; influence of the macrofungus *Ganoderma lucidum*



Rys. 2. Zmiana wartości PIRG w czasie dla wybranych patogenicznych grzybów i bakterii dla roślin pomidora; wpływ grzyba wielkowocznikowego *Pleurotus ostreatus*

Fig. 2. Change in PIRG value over time for selected fungal and bacterial pathogens for tomato plants; influence of the macrofungus *Pleurotus ostreatus*

-fungus interactions. This particular combination yielded a remarkably distinct result. It is noteworthy that, in the case of *P. viridilivida*, the use of macrofungi exhibited the opposite effect, actually stimulating bacterial growth.

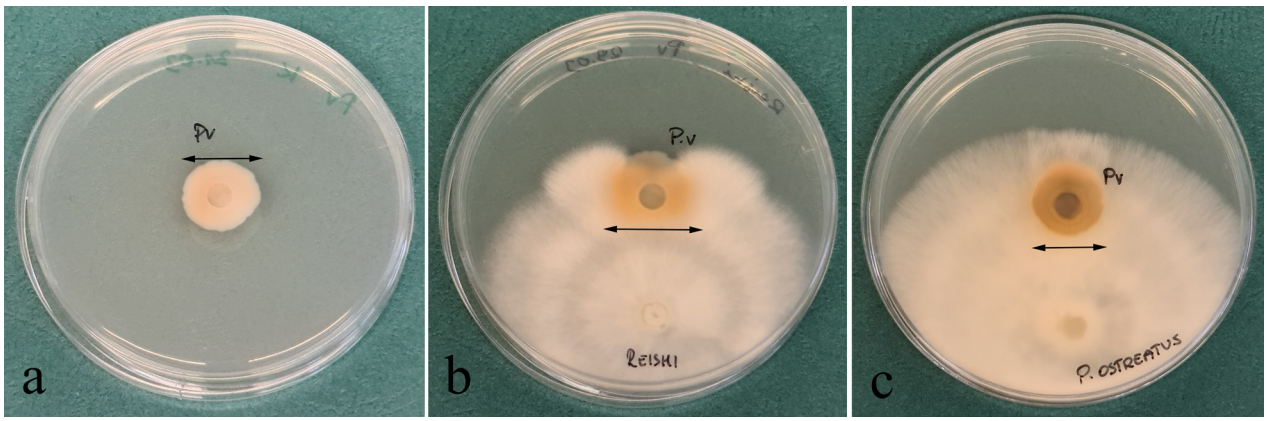
In both tests with *G. lucidum* and *P. ostreatus*, *P. viridilivida* exhibited more robust growth, particularly in the initial incubation period, compared to the growth of the control colony. However, after a week, the bacterial growth ceased (tab. 5, photo 4–6, fig. 1, 2). Photo 7 shows control growth of both *G. lucidum* and *P. ostreatus*.

The objective of this study was to examine the impact of the macrofungi mycelium *G. lucidum* and *P. ostreatus* on pathogens prevalent in tomato crops. We investigated whether and to what extent the mycelium of these macrofungi inhibits the growth of fungal and bacterial pathogens affecting tomato plants using the dual-culture test (fungi) and Weller's method (bacteria). The selected pathogenic fungi are well-known and commonly occurring in tomato crops. *Alternaria solani* causes early blight of tomatoes, characterized by stem rot, fruit damage, and leaf blight, affecting plants at all developmental stages (Kumar et al. 2018; Attia et al. 2020); *B. cinerea*, causing gray mold and infesting many other plants (Jin and Wu 2015; Wakeham et al. 2016), in the case of greenhouse tomatoes, prominent symptoms are observed on stems in areas of cut wounds, allowing the fungus to enter and cause stem rot. Soft rot of ripe tomato fruit mainly occurs after harvest (Williamson et al. 2007); and *F. oxysporum*, the causative agent of fusarium wilt in tomatoes, infects the vascular vessels, resulting in wilting, yellowing, and leaf drop (McGovern 2015; Worku and Sahe 2018). We specifically chose one of the most perilous and destructive tomato pathogens, *C. michiganensis*

Tabela 5. Procentowe zahamowanie rozrostu wybranych bakterii patogenicznych w ciągu kolejnych dni pomiaru w obecności grzybni grzybów wielkoowocnikowych (PIRG)

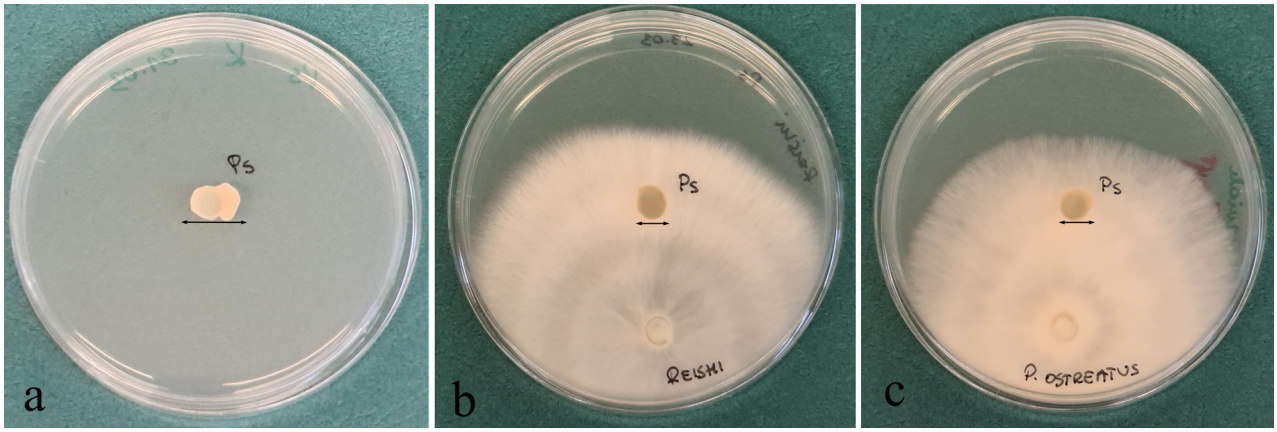
Table 5. Percentage inhibition of radial growth of selected pathogenic bacteria during the following measurement days in the presence of macrofungi (PIRG)

I pomiar (3. dzień inkubacji) I measurement (3th day of incubation)	*Pseudomonas viridilivida*	*Pseudomonas syringae*	*Clavibacter michiganensis* subsp. *michiganensis*
Ganoderma lucidum	–5.17%	15.9%	12.72%
Pleurotus ostreatus	–6.8%	20.45%	12.72%
II pomiar (5. dzień inkubacji) II measurement (5th day of incubation)	*Pseudomonas viridilivida*	*Pseudomonas syringae*	*Clavibacter michiganensis* subsp. *michiganensis*
Ganoderma lucidum	–5.47%	23.95%	25.35%
Pleurotus ostreatus	–9.5%	22.91%	12.67%
III pomiar (7. dzień inkubacji) III measurement (7th day of incubation)	*Pseudomonas viridilivida*	*Pseudomonas syringae*	*Clavibacter michiganensis* subsp. *michiganensis*
Ganoderma lucidum	9.09%	20.4%	34.56%
Pleurotus ostreatus	9.09%	24.48%	16.04%



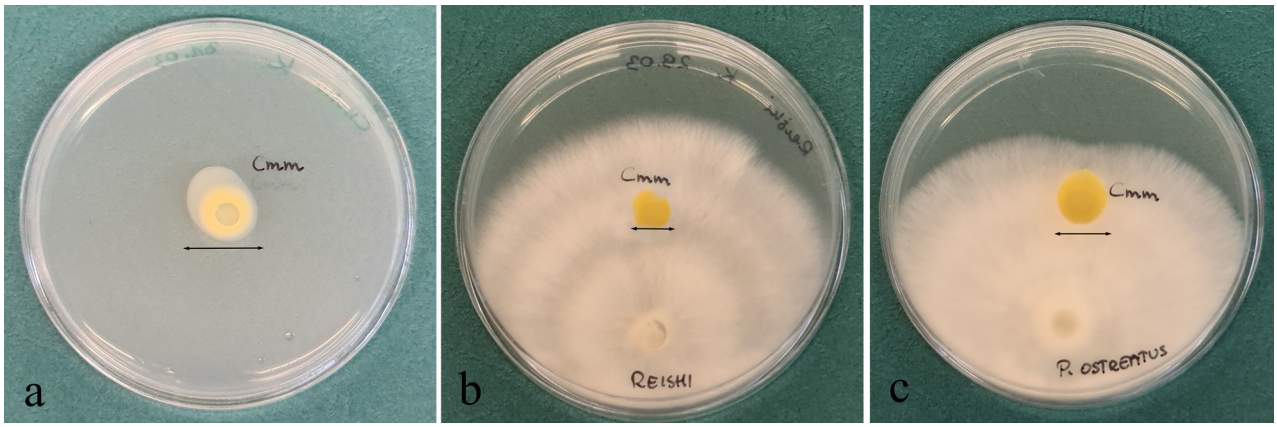
Fot. 4. Metoda Wellera: (a) wzrost kontrolny kolonii *Pseudomonas viridilivida*, (b) wzrost kolonii *Pseudomonas viridilivida* w obecności *Ganoderma lucidum*, (c) wzrost kolonii *Pseudomonas viridilivida* w obecności *Pleurotus ostreatus*; pomiar po 7 dniach

Photo 4. Weller's method test: (a) control growth of *Pseudomonas viridilivida* colony, (b) growth of *Pseudomonas viridilivida* colony in the presence of *Ganoderma lucidum*, (c) growth of *Pseudomonas viridilivida* colony in the presence of *Pleurotus ostreatus*; measurement after 7 days



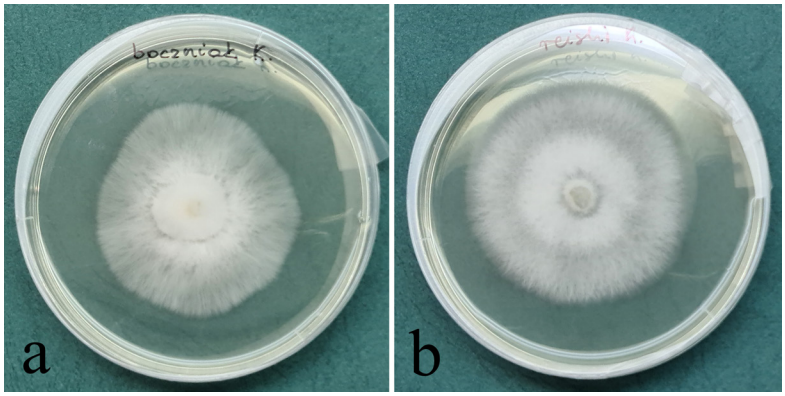
Fot. 5. Metoda Wellera: (a) wzrost kontrolny kolonii *Pseudomonas syringae*, (b) wzrost kolonii *Pseudomonas syringae* w obecności *Ganoderma lucidum*, (c) wzrost kolonii *Pseudomonas syringae* w obecności *Pleurotus ostreatus*; pomiar po 7 dniach

Photo 5. Weller's method test: (a) control growth of *Pseudomonas syringae* colony, (b) growth of *Pseudomonas syringae* colony in the presence of *Ganoderma lucidum*, (c) growth of *Pseudomonas syringae* colony in the presence of *Pleurotus ostreatus*; measurement after 7 days



Fot. 6. Metoda Wellera: (a) wzrost kontrolny kolonii *Clavibacter michiganensis* subsp. *michiganensis*, (b) wzrost kolonii *Clavibacter michiganensis* subsp. *michiganensis* w obecności *Ganoderma lucidum*, (c) wzrost kolonii *Clavibacter michiganensis* subsp. *michiganensis* w obecności *Pleurotus ostreatus*; pomiar po 7 dniach

Photo 6. Weller's method test: (a) control growth of *Clavibacter michiganensis* subsp. *michiganensis* colony, (b) growth of *Clavibacter michiganensis* subsp. *michiganensis* colony in the presence of *Ganoderma lucidum*, (c) growth of *Clavibacter michiganensis* subsp. *michiganensis* colony in the presence of *Pleurotus ostreatus*; measurement after 7 days



Fot. 7. Wzrost kontrolny grzybni: (a) *Pleurotus ostreatus*, (b) *Ganoderma lucidum*; pomiar po 7 dniach

Photo 7. Control growth of: (a) *Pleurotus ostreatus* mycelium, (b) *Ganoderma lucidum* mycelium; measurement after 7 days

subsp. *michiganensis*, responsible for bacterial canker. This plant vascular bacterium typically invades and proliferates in the xylem through natural openings or wounds, inducing symptoms of wilting and canker. The occurrence of asymptomatic latent infection and the invasion of tomato seeds by this bacterium are common occurrences (Gartemann et al. 2003; Nandi et al. 2018; Méndez et al. 2020). Additionally, we included the less recognized but potentially causing substantial losses, *P. syringae*, responsible for bacterial speck on tomatoes. Infections by this bacterium manifest as dark brown spots on leaves and fruits, measuring 1–3 mm in diameter, often accompanied by a yellow chlorotic halo (Kozik 2002; Zhao et al. 2003). We also opted for *P. viridilivida*, despite its infrequent mention in publications and studies. Interestingly, it was the third most commonly diagnosed pathogenic bacterium in tomato plant samples received by the Plant Disease Clinic and Bank of Plant Pathogen at the Institute of Plant Protection – National Research Institute in Poznań between 2013 and 2019, following *C. michiganensis* subsp. *michiganensis* and *P. syringae* (Zenelt et al. 2021).

Amid increasing concerns about the adverse effects of chemical plant protection products on the environment and human health, the quest for alternative methods of plant pest control has become imperative. Biological approaches utilizing natural organisms or their products offer a promising and sustainable alternative in agriculture. Macrofungi, such as *G. lucidum* (*reishi*) and *P. ostreatus* (*oyster mushroom*), are gaining considerable attention as potential sources of bioactive compounds with the ability to counteract plant infections. Research indicates that these fungi possess diverse antibacterial, antiviral, and antifungal properties, positioning them as promising candidates for use in controlling plant pathogens. Numerous studies have been conducted on the utilization of *G. lucidum* against well-known fungal pathogens, with a slightly smaller body of research focusing on its efficacy against bacterial plant pathogens. The primary compounds produced by *G. lucidum* encompass terpenoids (approximately 130 different types), triterpenes (e.g., ganodermic acid), polysaccharides (over 100 types, including mannitol and β-glucan), enzymes, proteins, lipids, phenols, and sterols (Kües et al. 2015; Cör et al. 2018; Sharma et al. 2019). Ganodermin, a protein isolated from *G. lucidum*, exhibits antifungal properties by inhibiting the growth of pathogens like *B. cinerea*, *F. oxysporum*, and other fungal species.

Several reports highlight the antagonistic effects of *G. lucidum* extracts (e.g., aqueous, ethanolic, and methanolic) against various fungi, including *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp. (Baig et al. 2015; Radhika and Rajan 2021), *Colletotrichum* sp. (Saludares et al. 2023), *Alternaria* sp. (Chen and Huang 2011; Shahid et al. 2016) and notably, *A. solani* (Asif et al. 2022). In our experiment, both *G. lucidum* and *P. ostreatus* notably inhibited *A. solani* and *F. oxysporum*. Similar outcomes with *G. lucidum* were observed by Saludares et al. (2023), who tested the antimicrobial properties of the fungus's extract against known plant pathogens, including *A. solani* and *F. oxysporum*, as well as by Baig et al. (2015). In both studies, the authors obtained results affirming the inhibitory properties of *G. lucidum* against these pathogens. Pavlov et al. (2021) reported antifungal activity of various *G. lucidum* strains against tested plant pathogenic fungi, with Basidiomycetes (*Heterobasidion* sp.) and Ascomycetes (*Bipolaris* sp., *Alternaria* sp., and *Fusarium* sp.) being the most sensitive phytopathogens. While the majority of such studies establish *G. lucidum*'s antifungal properties, there is also substantial literature supporting its antibacterial properties, particularly against bacteria pathogenic to humans and animals (Quereshi et al. 2010; Kamra and Bhatt 2012; Pala et al. 2019; Rezghi Jahromi and Mozafary 2021). Bacteriostatic properties against bacterial phytopathogens have been investigated by researchers such as Robles-Hernández et al. (2021), who examined culture fluids from *G. lucidum* against selected phytopathogenic bacteria. Similarly, Kamble et al. (2011) conducted a study testing various types of extracts (methanol, acetone, water, chloroform) and their effects on bacterial pathogens.

Similarly, *P. ostreatus*, renowned for its potent antioxidant and antibacterial potential, may possess attributes that limit the development of plant pathogens. Pleurostrin, a peptide with antifungal properties analogous to ganodermin, has been isolated from this species (Wang and Ng 2006). Although there are few reports about the use of *P. ostreatus* against plant pathogens, there are instances where it has been highly effective in inhibiting the growth of pathogens such as *B. sorokiniana*, *F. culmorum*, or *R. cerealis*. In turn, Ocimati et al. (2021) explored the potential of spent *P. ostreatus* substrate to inhibit *Fusarium* spp. in banana cultivation, suggesting its use as a practice to reduce Fusarium-related diseases in banana cultivation. To the best of our knowledge, this is the first instance where *P. ostreatus* mycelium has been utilized to assess its antagonistic properties against pathogenic bacteria affecting tomato plants. Due to the limited number of reports on the use of this macrofungus against phytopathological bacteria, specific examples for comparison with our results are unavailable.

The majority of research focuses on the impact of the *oyster mushroom* on bacterial human pathogens, such as *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* (Vamanu 2013; Al-Bahrani et al. 2017; Ianni et al. 2021). This fungus exhibits a broad spectrum of antibacterial properties (Lesa et al. 2022), suggesting its potential antagonistic effect against phytopathogenic bacteria. In our studies, *P. ostreatus* demonstrated a less effective antifungal and antibacterial impact compared to *G. lucidum*. Among fungal pathogens, *A. solani* was the most strongly inhibited,

with linear inhibition throughout the entire incubation period. For *B. cinerea*, initial inhibition was observed, but after 6 days of incubation, *B. cinerea* significantly accelerated its growth. By the end of the incubation, however, the growth of the pathogen mycelia was halted. Concerning bacteria, noticeable growth inhibition was observed for *C. michiganensis* subsp. *michiganensis* and *P. syringae*. An intriguing phenomenon was the observation of *P. ostreatus* stimulating the growth of *P. viridilivida*, contrary to our expectations. Further research is necessary to determine the factors causing this unexpected phenomenon. In natural environments, numerous interactions occur among different groups of microorganisms. It is possible that *P. ostreatus* created favorable conditions for the development of *P. viridilivida*, perhaps by providing nutrients conducive to bacterial growth or transforming available organic substances in the substrate into forms beneficial for bacteria.

It is crucial to note that *G. lucidum*, despite being globally recognized for its health-promoting properties, is also acknowledged as a pathogen that can lead to substantial losses in certain plants (Fernando 2008; Deepatharshini and Elango 2015; Shah et al. 2021). Phytopathogenic species within the *Ganoderma* genus can induce severe diseases such as stem, trunk, and root rot, particularly affecting economically significant trees and perennial crops, especially in tropical regions (Deepatharshini and Elango 2015; Kües et al. 2015). In India, the decline in productivity and plant mortality, including oil palm, coconut, and tea, are significant economic consequences of diseases caused by *Ganoderma*. This fungus is identified as a serious pathogen affecting crops, forest plantations, and trees in natural forests in the country (Sankaran et al. 2005). Losses primarily stem from the fungus's ability to decompose wood components, including lignin, cellulose, and hemicellulose, resulting in wood rot (Piętka and Byk 2018). Similarly, *P. ostreatus* is implicated in wood rot and can pose a threat to old and weakened trees, such as beech, alder, or oak (Karim et al. 2016; Bari et al. 2017, 2020).

The antagonistic effect of macrofungi, specifically *G. lucidum* and *P. ostreatus*, against pathogens may involve diverse mechanisms influenced by factors such as the chemical composition and metabolites of these fungi. Therefore, further research is imperative to gain a deeper understanding of how *G. lucidum* and *P. ostreatus* impact pathogens in tomato plants and how their potential can be harnessed to promote healthier and more resilient crops. Our research endeavors to contribute to the development of ecological and sustainable strategies for plant infection control. By safeguarding crops from harmful pathogens, we aim to minimize negative impacts on both the environment and human health. This pursuit aligns with the broader goal of fostering agricultural practices that are not only effective but also environmentally conscious and conducive to long-term crop sustainability.

Literatura / References

Al-Bahrani R., Raman J., Lakshmanan H., Hassan A.A., Sabaratnam V. 2017. Green synthesis of silver nanoparticles using tree oyster mushroom *Pleurotus ostreatus* and its inhibitory activity against pathogenic bacteria. Materials Letters 186 (1): 21–25. DOI: 10.1016/j.matlet.2016.09.069

Asif M., Shahid A., Ahmad N. 2022. *Ganoderma lucidum* as a biocontrol agent for management of *Alternaria solani*, a pathogen of early blight of tomato. Sarhad Journal of Agriculture 38 (2): 734–741. DOI: 10.17582/journal.sja/2022/38.2.734.741

Atri N., Sharma S.K., Joshi R., Gulati A., Gulati A. 2013. Nutritional and neutraceutical composition of five wild culinary-medicinal species of genus *Pleurotus* (higher Basidiomycetes) from northwest India. International Journal of Medicinal Mushrooms 15 (1): 49–56. DOI: 10.1615/intjmedmushr.v15.i1.60

Attia M.S., El-Sayyad G.S., Abd Elkodous M., El-Batal A.I. 2020. The effective antagonistic potential of plant growth-promoting rhizobacteria against *Alternaria solani*-causing early blight disease in tomato plant. Scientia Horticulturae 266: 109289. DOI: 10.1016/j.scienta.2020.109289

Baig M.N., Shahid A.A., Ali M. 2015. In vitro assessment of extracts of the lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (higher Basidiomycetes) against different plant pathogenic fungi. International Journal of Medicinal Mushrooms 17 (4): 407–411. DOI: 10.1615/intjmedmushrooms.v17.i4.90

Bari E., Daniel G., Yilgor N. 2020. Comparison of the decay behavior of two white-rot fungi in relation to wood type and exposure conditions. Microorganisms 8 (12): 1931. DOI: 10.3390/microorganisms8121931

Bari E., Oladi M.K.R., Ghanbary M.A.T., Daryaei M.G., Benz O.S.J.P. 2017. A comparison between decay patterns of the white-rot fungus *Pleurotus ostreatus* in chestnut – leaved oak (*Quercus castaneifolia*) shows predominantly simultaneous attack both in vivo and in vitro. Forest Pathology 47 (4): e12338. DOI: 10.1111/efp.12338

Bertini L., Amicucci A., Agostini D. 1999. A new pair of primers designed for amplification of the ITS region in Tuber species. FEMS Microbiology Letters 173 (1): 239–245. DOI: 10.1111/j.1574-6968.1999.tb13508.x

Bhardwaj K., Sharma A., Tejwan N. 2020. *Pleurotus* macrofungi-assisted nanoparticle synthesis and its potential applications: a review. Journal of Fungi 6 (4): 351. DOI: 10.3390/jof6040351

Buell C.R., Joardar V., Lindeberg M. 2003. The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. Proceedings of the National Academy of Sciences of the United States of America 100 (18): 10181–10186. DOI: 10.1073/pnas.1731982100

Chaerani R., Voorrips R.E. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. Journal of General Plant Pathology 72: 335–347. DOI: 10.1007/s10327-006-0299-3

Chen J., Huang J. 2011. Antimicrobial activity of edible mushroom culture filtrates on plant pathogens. Plant Pathology Bulletin 19 (4): 261–270. DOI: 10.3390/app12094591

Cör D., Knez Ž., Hrnčič M.K. 2018. Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of *Ganoderma lucidum* terpenoids and polysaccharides: a review. Molecules 23 (3): 649. DOI: 10.3390/molecules23030649

da Cruz M.P., Mazaro S.M., Bruzamarello J. 2019. Bioactive compounds of *Ganoderma lucidum* activate the defense mechanisms of soybean plants and reduce the severity of powdery mildew. Journal of Agricultural Science 11 (13): 99–114. DOI: 10.5539/jas.v11n13p99

De Silva D.D., Rapior S., Fons F., Bahkali A.H., Hyde K.D. 2012. Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer effects and putative mechanisms of action. Fungal Diversity 55: 1–35. DOI: 10.1007/s13225-012-0151-3

Deepatharshini D., Elango A. 2015. Antifungal activity of leaf extract of *Eichhorinia crassipes* against *Ganoderma lucidum* causing basal stem rot disease in coconut tree. World Journal of Pharmacy and Pharmaceutical Sciences 4 (6): 859–864.

El Domany E.B., Essam T.M., Ahmed A.E., Farghali A.A. 2018. Biosynthesis physico-chemical optimization of gold nanoparticles as anti-cancer and synergetic antimicrobial activity using *Pleurotus ostreatus* fungus. Journal of Applied Pharmaceutical Science 8 (5): 119–128. DOI: 10.7324/JAPS.2018.8516

FAOSTAT 2021. https://www.fao.org/faostat/en/#data/QCL [dostęp: 29.02.2024].

Fernando K.M.E.P. 2008. The host preference of a *Ganoderma lucidum* strain for three tree species of Fabaceae family; *Cassia nodosa*, *Cassia fistula* and *Delonix regia*. Journal of the National Science Foundation of Sri Lanka 36 (4): 323–326. DOI: 10.4038/jnsfsr.v36i4.271

Fritz M., Jakobsen I., Lyngkjær M.F., Thordal-Christensen H., Pons-Kühnemann J. 2006. Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza 16 (6): 413–419. DOI: 10.1007/s00572-006-0051-z

Gartemann K.H., Kirchner O., Engemann J., Gräfen I., Eichenlaub R., Burger A. 2003. *Clavibacter michiganensis* subsp. *michiganensis*: first steps in the understanding of virulence of a gram-positive phytopathogenic bacterium. Journal of Biotechnology 106 (2–3): 179–191. DOI: 10.1016/j.jbiotec.2003.07.011

Gashaw G., Fassil A., Redi F. 2020. Evaluation of the antibacterial activity of *Pleurotus* spp. cultivated on different agricultural wastes in Chiro, Ethiopia. International Journal of Microbiology 2020: 9312489. DOI: 10.1155/2020/9312489

Gashaw G., Getu A. 2021. Phytochemical characteristics evaluation of *Pleurotus* species cultivated on agricultural wastes in Chiro, Ethiopia. Indian Journal of Agricultural Research 55 (3): 289–295. DOI: 10.18805/IJARe.A-526

Ge B.B., Cheng Y., Liu Y., Liu B.H., Zhang K.C. 2015. Biological control of *Botrytis cinerea* on tomato plants using *Streptomyces ahygroscopicus* strain CK-15. Letters in Applied Microbiology 61 (6): 596–602. DOI: 10.1111/lam.12500

Grys A., Hołderna-Kędzia E., Łowicki Z. 2011. *Ganoderma lucidum* – grzyb o cennych właściwościach farmakologicznych. [*Ganoderma lucidum* – fungus with valuable pharmacological properties]. Postępy Fitoterapii 1/2011: 28–33.

Hall T.A. 1999. BioEdit: a user friendly biological seque alignment aditor and analysis program for Windows 95/98/NT. Nucleid Acids Symposium Series 41: 95–98.

Ianni F., Blasi F., Angelini P. 2021. Extraction optimization by experimental design of bioactives from pleurotus ostreatus and evaluation of antioxidant and antimicrobial activities. Processes 9 (5): 743. DOI: 10.3390/pr9050743

Jin W., Wu F. 2015. Characterization of miRNAs associated with *Botrytis cinerea* infection of tomato leaves. BMC Plant Biology 15 (1): 1–14. DOI: 10.1186/s12870-014-0410-4

Kamble R., Venkata S., Gupte A.M. 2011. Antimicrobial activity of *Ganoderma lucidum* mycelia. Journal of Pure and Applied Microbiology 5 (2): 983–986.

Kamra A., Bhatt A.B. 2012. Evaluation of antimicrobial and antioxidant activity of *Ganoderma lucidum* extracts against human pathogenic bacteria. International Journal of Pharmacy and Pharmaceutical Sciences 4 (2): 359–362. DOI: 10.12691/jfnr-2-8-1

Karim M., Daryaei M.G., Torkaman J., Oladi R., Ghanbary M.A.T., Bari E. 2016. *In vivo* investigation of chemical alteration in oak wood decayed by *Pleurotus ostreatus*. International Biodeterioration and Biodegradation 108: 127–132. DOI: 10.1016/j.ibiod.2015.12.012

Kozik E.U. 2002. Studies on resistance to bacterial speck (*Pseudomonas syringae* pv. *tomato*) in tomato cv. Ontario 7710. Plant Breeding 121 (6): 526–530. DOI: 10.1046/j.1439-0523.2002.00766.x

Kozik E.U., Sobiczewski P. 2000. Response of tomato genotypes to bacterial speck (*Pseudomonas syringae* pv. *tomato*). Acta Physiologiae Plantarum 22: 243–246. DOI: 10.1007/s11738-000-0021-6

Krupodorova T.A., Barshteyn V.Y., Zabeida E.F., Pokas E.V. 2016. Antibacterial activity of macromycetes mycelia and culture liquid. Microbiology and Biotechnology Letters 44 (3): 246–253. DOI: 10.4014/mbl.1603.03003

Kumar S., Singh R., Kashyap P.L., Srivastava A.K. 2013. Rapid detection and quantification of *Alternaria solani* in tomato. Scientia Horticulturae 151: 184–189. DOI: 10.1016/j.scienta.2012.12.026

Kumar S.P., Srinivasulu A., Raja Babu K. 2018. Symptomology of major fungal diseases on tomato and its management. Journal of Pharmacognosy and Phytochemistry 7 (6): 1817–1821. DOI: 10.26420/austinjplantbiol.2023.1035

Kumar V., Yadav U. 2014. Screening of antifungal activity of *Pleurotus ostreatus* and *Agaricus bisporus*. Biolife 2 (3): 918–923. DOI: 10.13140/RG.2.2.18735.87205

Kües U., Nelson D.R., Liu C. 2015. Genome analysis of medicinal *Ganoderma* spp. with plant-pathogenic and saprotrophic life-styles. Phytochemistry 114: 18–37. DOI: 10.1016/j.phytochem.2014.11.019

Lesa K.N., Khandaker M.U., Iqbal F.M.R., Sharma R., Islam F., Mitra S., Emran T.B. 2022. Nutritional value, medicinal importance, and health-promoting effects of dietary mushroom (*Pleurotus ostreatus*). Journal of Food Quality 2022: 2454180. DOI: 10.1155/2022/2454180

Mansfield J., Genin S., Magori S. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Molecular Plant Pathology 13 (6): 614–629. DOI: 10.1111/j.1364-3703.2012.00804.x

McGovern R.J. 2015. Management of tomato diseases caused by *Fusarium oxysporum*. Crop Protection 73: 78–92. DOI: 10.1016/j.cropro.2015.02.021

Méndez V., Valenzuela M., Salvà-Serra F., Jaén-Luchoro D., Besoain X., Moore E.R.B., Seeger M. 2020. Comparative genomics of pathogenic *Clavibacter michiganensis* subsp. *michiganensis* strains from chile reveals potential virulence features for tomato plants. Microorganisms 8 (11): 1679. DOI: 10.3390/microorganisms8111679

Moradali M.F., Mostafavi H., Ghods S., Hedjaroude G.A. 2007. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). International Immunopharmacology 7 (6): 701–724. DOI: 10.1016/j.intimp.2007.01.008

Mustafin K., Bisko N., Blieva R., Al-Maali G., Krupodorova T., Narmuratova Z., Saduyeva Z., Zhakipbekova A. 2022. Antioxidant and antimicrobial potential of *Ganoderma lucidum* and *Trametes versicolor*. Turkish Journal of Biochemistry 47 (4): 483–489. DOI: 10.1515/tjb-2021-0141

Nandi M., Macdonald J., Liu P., Weselowski B., Yuan Z.C. 2018. *Clavibacter michiganensis* ssp. *michiganensis*: bacterial canker of tomato, molecular interactions and disease management. Molecular Plant Pathology 19 (8): 2036–2050. DOI: 10.1111/mpp.12678

Ocimati W., Were E., Tazuba A.F., Dita M., Zheng S., Blomme G. 2021. Spent *Pleurotus ostreatus* substrate has potential for managing Fusarium wilt of banana. Journal of Fungi 7 (11): 946. DOI: 10.3390/jof7110946

Ofodile L.N., Ogbe A.O., Oladipupo O. 2011. Effect of the mycelial culture of *Ganoderma lucidum* on human pathogenic bacteria. International Journal of Biology 3 (2): 111–114. DOI: 10.5539/ijb.v3n2p111

Owaid M.N., Al-Saeedi S., Al-Assaffii I.A. 2015. Antimicrobial activity of mycelia of oyster mushroom species (*Pleurotus* spp.) and their liquid filtrates (*in vitro*). Journal of Medical and Bioengineering 4 (5): 376–380. DOI: 10.12720/jomb.4.5.376-380

Pala S.A., Wani A.H., Ganai B.A. 2019. Antimicrobial potential of some wild macromycetes collected from Kashmir Himalayas. Plant Science Today 6 (2): 137–146. DOI: 10.14719/pst.2019.6.2.503

Parola S., Chiodaroli L., Orlandi V., Vannini C., Panno L. 2017. Lentinula edodes and *Pleurotus ostreatus*: functional food with antioxidant – antimicrobial activity and an important source of vitamin D and medicinal compounds. Functional Foods in Health and Disease 7 (10): 773–794. DOI: 10.31989/ffhd.v7i10.374

Pavlov I.N., Litovka Y.A., Makolova P.V., Timofeev A.A., Litvinova E.A., Enazarov R.K. 2021. Prospects for using *Ganoderma lucidum* (Curtis) P. Karst. for biological control of phytopathogenic fungi. IOP Conference Series: Earth and Environmental Science 848 (1): 012162. DOI: 10.1088/1755-1315/848/1/012162

Piętka J., Byk A. 2018. Rozkład drewna olszy czarnej *Alnus glutinosa* (L.) Gaertn. przez grzybnię lakownicy żółtawej *Ganoderma lucidum* (Curtis) P. Karst. w warunkach laboratoryjnych. [Decay of black alder *Alnus glutinosa* (L.) Gaertn. wood by myceliumof *Ganoderma lucidum* (Curtis) P. Karst. in laboratory conditions]. Sylwan 162 (2): 138–145.

Quereshi S., Pandey A.K., Sandhu S.S. 2010. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. People's Journal of Scientific Research 3 (1): 9–13.

Radhika R., Rajan S. 2021. Antifungal potentials of *Ganoderma lucidum* extracts. Plant Cell Biotechnology and Molecular Biology 22 (37–38): 22–27.

Raman J., Jang K.Y., Oh Y.L. 2021. Cultivation and nutritional value of prominent *Pleurotus* spp.: an overview. Mycobiology 49 (1): 1–14. DOI: 10.1080/12298093.2020.1835142

Rezghi Jahromi M.H., Mozafary M. 2021. *Ganoderma lucidum* and antimicrobial activity. Journal of Ethno-Pharmaceutical Products 2 (2): 35–41.

Robles-Hernández L., Salas-Salazar N.A., Gonzalez-Franco A.C. 2021. Purification and characterization of antibacterial activity against phytopathogenic bacteria in culture fluids from *Ganoderma lucidum*. Molecules 26 (18): 5553. DOI: 10.3390/molecules26185553

Saludares G.G., Amper C.D., Lituanas I.M. 2023. Antimicrobial performances of *Ganoderma lucidum* extract against fruits and leaves pathogens. IOP Conference Series: Earth and Environmental Science 1145 (1): 012019. DOI: 10.1088/1755-1315/1145/1/012019

Sankaran K., Bridge P.D., Gokulapalan C. 2005. *Ganoderma* diseases of perennial crops in India – an overview. Mycopathologia 159: 143–152. DOI: 10.1007/s11046-004-4437-1

Seweryn E., Ziała A., Gamian A. 2021. Health-promoting of polysaccharides extracted from *Ganoderma lucidum*. Nutrients 13 (8): 2725. DOI: 10.3390/nu13082725

Shah K.K., Tiwari I., Modi B., Pandey H.P., Subedi S., Shrestha J. 2021. Shisham (*Dalbergia sissoo*) decline by dieback disease, root pathogens and their management: a review. Journal of Agriculture and Natural Resources 4 (2): 255–272. DOI: 10.3126/janr.v4i2.33915

Shahid A.A., Asif M., Shahbaz M., Ali M. 2016. Antifungal potential of *Ganoderma lucidum* extract against plant pathogenic fungi of *Calendula officinalis* L. 5th International Conference on Biological, Chemical and Environmental Sciences (BCES-2016), March 24–25, 2016, London (UK). DOI: 10.15242/IICBE.C0316005

Sharma C., Bhardwaj N., Sharma A. 2019. Bioactive metabolites of *Ganoderma lucidum*: factors, mechanism and broad spectrum therapeutic potential. Journal of Herbal Medicine 17–18: 100268. DOI: 10.1016/j.hermed.2019.100268

Sonawane A., Mahajan M., Renake S. 2015. Antifungal activity of a fungal isolates against *Pomegranate* wilt pathogen *Fusarium.* International Journal of Current Microbiology and Applied Sciences 4 (2): 48–57.

Srinivas C., Nirmala Devi D., Narasimha Murthy K. 2019. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: biology to diversity – a review. Saudi Journal of Biological Sciences 26 (7): 1315–1324. DOI: 10.1016/j.sjbs.2019.06.002

Takken F., Rep M. 2010. The arms race between tomato and *Fusarium oxysporum*. Molecular Plant Pathology 11 (2): 309–314. DOI: 10.1111/j.1364-3703.2009.00605.x

Valenzuela M., Besoain X., Durand K. 2018. *Clavibacter michiganensis* subsp. *michiganensis* strains from central Chile exhibit low genetic diversity and sequence types match strains in other parts of the world. Plant Pathology 67 (9): 1944–1954. DOI: 10.1111/ppa.12911

Vamanu E. 2013. Studies on the antioxidant and antimicrobial activities of *Pleurotus ostreatus* PSI101109 mycelium. Pakistan Journal of Botany 45 (1): 311–317.

Vancov T., Keen B. 2009. Amplification of soil fungal community DNA using the ITS86F and ITS4 primers. FEMS Microbiology Letters 296 (1): 91–96. DOI: 10.1111/j.1574-6968.2009.01621.x

Wakeham A., Langton A., Adams S., Kennedy R. 2016. Interface of the environment and occurrence of *Botrytis cinerea* in pre-symptomatic tomato crops. Crop Protection 90: 27–33. DOI: 10.1016/j.cropro.2016.08.014

Waktola G., Temesgen T. 2020. Pharmacological activities of oyster mushroom (*Pleurotus ostreatus*). Novel Research in Microbiology Journal 4 (2): 688–695. DOI: 10.21608/nrmj.2020.84017

Wang H., Ng T.B. 2006. Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. Peptides 27 (1): 27–30. DOI: 10.1016/j.peptides.2005.06.009

Weller D.M., Zhang B.X., Cook R.J. 1985. Application of a rapid screening test for selection of bacteria suppressive to take-all of wheat. Plant Disease 69 (8): 710–713.

Williamson B., Tudzynski B., Tudzynski P., Van Kan J.A.L. 2007. *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology 8 (5): 561–580. DOI: 10.1111/j.1364-3703.2007.00417.x

Worku M., Sahe S. 2018. Review on disease management practice of tomato wilt caused *Fusarium oxysporum* in case of Ethiopia. Journal of Plant Pathology and Microbiology 9 (11): 9–12. DOI: 10.4172/2157-7471.1000460

Younis A.M., Wu F.S., El Shikh H.H. 2015. Antimicrobial activity of extracts of the oyster culinary medicinal mushroom *Pleurotus ostreatus* (higher basidiomycetes) and identification of a new antimicrobial compound. International Journal of Medicinal Mushrooms 17 (6): 579–590. DOI: 10.1615/IntJMedMushrooms.v17.i6.80

Zenelt W., Krawczyk K., Borodynko-Filas N. 2021. Biodiversity and scope of endophytic and phytopathogenic bacterial species identified in plant samples investigated in the Plant Disease Clinic laboratory. Journal of Plant Protection Research 61 (1): 63–82. DOI: 10.24425/jppr.2021.136274

Zhao Y., Thilmony R., Bender C.L., Schaller A., He S.Y., Howe G.A. 2003. Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. Plant Journal 36 (4): 485–499. DOI: 10.1046/j.1365-313x.2003.01895.x