7th International Congress “Engineering, Environment and Materials in Process Industry” Jahorina, March 17th – 19th 2021

We would like to announce that the 7th International Congress *Engineering, Environment and Materials in Process Industry*, EEM2021, was successfully held at mountain of Jahorina, from March 17th to March 19th, 2021. The EEM2021 congress gathered eminent researchers and professionals from over 30 countries. A total of 228 papers were submitted, and the fields of greatest interest were chemical engineering and technology, food technology, biotechnology, materials and environmental sciences. This year’s event was supported by two internationally renowned scientific publishers – *Springer-Nature* and *Wiley*, who were kind enough to offer their scientific journals to the research results presented, together with other renowned EEM2021 supporting publications – *SN Applied Sciences, Journal of Food Processing and Preservation, Health, Food & Biotechnology, Hemijska industrija, and Journal of Engineering & Processing Management.* Due to the COVID-19 outbreak, we were forced to adapt to this new situation and this was first congress in the history of our institution that was conducted as a hybrid event – both in person and on a digital platform. This mode of organization enabled participants from all over the world to attend plenary lectures and invited speakers’ presentations, as well as to participate in an online poster session. Since all congress events were streamed live on YouTube, those who were not able to attend EEM2021 online can watch the whole event here:

https://www.youtube.com/watch?v=ymgFg8FlkqQ

https://www.youtube.com/watch?v=aN5jEvGcHFI

https://www.youtube.com/watch?v=CvUD6WOkYCM

The International Congress *Engineering, Environment and Materials in Process Industry* once again proved to be an opportunity for members of the scholarly community to exchange the results of their work internationally and gain insight into the possibilities of applying their research results in practice, despite the ongoing challenges of the modern world. The Organizing Committee and members of Faculty of Technology Zvornik would like to thank all the researchers, delegates, reviewers, endorsers, co-organizers and sponsors for their major contribution to EEM2021. We would also like to invite all researchers to take part in our next congress, which will be held hopefully under much more fortunate conditions, and the Organizing Committee will do their best to outdo themselves and make the following event better in every manner.

Plenary Lecturers

- George Dedoussis, *Harokopio University of Athens, School of Health Science and Education, Athens, Greece*
- Filip Kokalj, *University of Maribor, Faculty of Mechanical Engineering, Maribor, Slovenia*
- Ivana Smičiklas, *University of Belgrade, Vinča Institute of Nuclear Sciences, Belgrade, Serbia*
- Uroš Cvelbar, *Jožef Stefan Institute, Ljubljana, Slovenia*
- Dejan Popović *Serbian Academy of Sciences and Arts (SASA), Belgrade, Serbia*
- Constantinos Georgiou *Agricultural University of Athens, Chemistry Laboratory, Athens, Greece*
- Apostolis Koutinas *Agricultural University of Athens, Department of Food Science and Technology, Athens, Greece*

Topics

- *Chemical and Electrochemical Engineering*
- *Food Engineering and Biotechnology*
- *Environmental Engineering*
- *Materials and Material Characterization*
- *Nanotechnology*
- *Inorganic Chemistry and Technology*
- *Organic Chemistry and Technology, Polymers*
- *Plasma Technology*
- *Energy Efficiency and Renewable Energy Sources*
- *Textile Engineering*
- *Corrosion and Protection of Materials and Thermal Power Plants*
- *Metallurgy*
- *Management in the Process Industry*
- *General Sections*

VII International Congress

"Engineering, Environment and Materials in Process Industry"
EEM2021

March 17-19, 2021

Jahorina, Republic of Srpska, Bosnia and Herzegovina

CONGRESS PROGRAM

Wednesday, March 17, 2021

10:00 – 20:00	REGISTRATION (*Termag* Hotel)
15:30 – 16:00	OPENING CEREMONY
16:00 – 16:45	***PLENARY LECTURE*** **George Dedoussis**, *Harokopio University of Athens, School of Health Science and Education, Athens, Greece* – **MASTIHA TREATMENT FOR OBESE WITH NAFLD DIAGNOSIS. THE MAST4HEALTH EU PROGRAM**
16:45 – 17:00	COFFEE BREAK
17:00 – 17:45	***PLENARY LECTURE*** **Ivana Smičiklas**, *University of Belgrade, Vinča Institute of Nuclear Sciences, Belgrade, Serbia* – **VALORIZING SECONDARY SOURCES OF PHOSPHORUS: APPLICABILITY OF BIOLOGICAL APATITE IN SOIL REMEDIATION AND REHABILITATION**
18:00	COCTAIL RECEPTION
	DINNER

Thursday, March 18, 2021

09:00 – 09:45	***PLENARY LECTURE*** **Filip Kokalj**, *University of Maribor, Faculty of Mechanical Engineering, Maribor, Slovenia* – **WASTE-TO-ENERGY IN THE LIGHT OF CIRCULAR ECONOMY AND EU WASTE MANAGEMENT PLANS**
09:45 – 10:30	***PLENARY LECTURE*** **Dejan Popović**, *Serbian Academy of Sciences and Arts (SASA), Belgrade, Serbia* – **WHERE THE ENGINEERING MEETS THE NATURE: EXTERNAL CONTROL OF SENSORY-MOTOR SYSTEMS WITH DISABILITY**
10:30 – 11:00	***PRESENTATION*** **Suzana Gotovac Atlagić**, *Bosnia and Herzegovina representative in the COST Scientific Committee* – **IMPORTANCE AND METHODOLOGY OF PARTNER AND SHARING PARTICIPATION OF THE INCLUSIVE COUNTRIES IN COST ASSOCIATION-SUPPORTED ACTIONS**
11:00 – 11:30	COFFEE BREAK
11:30 – 12:15	***PLENARY LECTURE*** **Uroš Cvelbar**, *Jožef Stefan Institute, Ljubljana, Slovenia* – **PLASMA AS A TOOL FOR DECONTAMINATION OF NATURAL TOXINS**
12:15 – 12:35	***INVITED LECTURE*** **Nikša Krstulović**, *Institute of Physics, Zagreb, Croatia* – **PHOTOCATALYTIC ACTIVITY OF LASER SYNTHESIZED TiO_2 AND ZnO NANOPARTICLES**
12:35 – 12:55	COFFEE BREAK
12:55 – 13:40	***PLENARY LECTURE*** **Constantinos Georgiou**, *Agricultural University of Athens, Chemistry Laboratory, Athens, Greece* – **ELEMENTAL METABOLOMICS**
13:40 – 14:00	SPONSOR PRESENTATION
14:00 – 16:00	LUNCH

16:00 – 16:20	***INVITED LECTURE*** **Kinga Kutasi**, *Institute for Solid State Physics and Optics, Wigner Research Centre for Physics, Budapest, Hungary* – **SURFACE-WAVE MICROWAVE DISCHARGE: A POSSIBLE TOOL IN PLASMA AGRICULTURE**
16:20 – 16:40	***INVITED LECTURE*** **Anet Režek Jambrak**, *Faculty of Food Technology and Biotechnology (PBF), University of Zagreb, Croatia* – **APPLICATION OF COLD PLASMA IN SUSTAINABLE FOOD PROCESSING**
16:40 – 17:00	**SPONSOR PRESENTATION**
17:00 – 18:00	**POSTER DISCUSSION AND SCIENTIFIC COMMITTEE MEETING**
	DINNER

Friday, March 19, 2021

09:30 – 10:15	***PLENARY LECTURE*** **Apostolis Koutinas**, *Agricultural University of Athens, Department of Food Science and Technology, Athens, Greece* – **BIOREFINERY DEVELOPMENT FOR SUSTAINABLE PRODUCTION OF BIO-BASED PRODUCTS WITHIN A CIRCULAR BIO-ECONOMY CONTEXT**
10:15 – 10:35	***INVITED LECTURE*** **Biljana Mijović**, *University of East Sarajevo, Faculty of Medicine, Foča, Republic of Srpska, Bosnia and Herzegovina* – **IMMUNOLOGICAL REACTION TO COAL DUST IN COAL WORKERS**
10:35 – 10:55	***INVITED LECTURE*** **Slađana Tanasković**, *University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia* – **MACROCYCLIC Co(II) AND Cu(II) COMPLEXES WITH ADDITIONAL DICARBOXILATE LIGANDS: ANTIMICROBIAL AND CYTOTOXIC PROPERTIES**
10:55 – 11:15	***INVITED LECTURE*** **Kandrokov Roman Khazhsetovich**, *Department of Grains, Bakery and Confectionery Technologies, Moscow State University of Food Production, Russia* – **INNOVATIVE TECHNOLOGY FOR PROCESSING FOOD GRAIN TRITICALE INTO VARIETAL FLOUR AND FLOUR FOR PASTA**
11:15 – 11:35	***INVITED LECTURE*** **Ahsen Rayman Ergün**, *Food Engineering Department, Faculty of Engineering, Ege University, Izmir, Turkey* – **OSMOTIC DEHYDRATION OF PINEAPPLE SLICES PRE-TREATED BY ELECTROPLASMOLYSIS: DETERMINATION OF COLOR CHANGE KINETICS**

11:35	**CLOSING CEREMONY**

UDK 573.4:633.15
DOI: 10.7251/EEMEN2101062M
Scientific paper

BIOCONTROL POTENTIAL OF DIFFERENT *TRICHODERMA* ISOLATES AGAINST MAIZE PHYTOPATHOGENS

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Abstract

Usage of chemical plant protection agents in agricultural production contributes to environmental pollution and emissions of factors harmful for human health. It has been noted that their overexploitation has resulted in the emergence of resistant species and chemical compounds in the food chain, which indicates the need to find new agents with improved characteristics. One of the solutions is certainly the use of beneficial microorganisms. Considering that maize is a significant agricultural crop very present in the human diet, its health safety is of great importance. In this paper, the ability of three Trichoderma isolates, isolated from the environment, in the biological control of the most common maize pathogens, was examined. Cultivation of Tricoderma isolates was performed on PDB (Potato Dextrose Broth) medium by shaking at 150 rpm for 7 days at 25°C. After 7 days of cultivation, the effect of both, cultivation broth and supernatant, against selected maize phytopathogens was examined using wells diffusion technique. The results showed that the cultivation broth showed a statistically significant efficacy on selected maize phytopathogens compared to the supernatant, using all Trichoderma isolates. By applying the Scheffe test, it was determined that the best effect on the test maize phytopathogens show cultivation broth of T. harzianum, forming maximum mean inhibition zone diameters of 50.33 mm for Fusarium graminearum, 40.67 mm for Helminthosporium carbonum, 25.67 mm for Aspergillus flavus and 25.33 mm for Penicillium sp. Since each inhibition zone larger than 22 mm shows that the produced agent is highly efficient, based on the obtained inhibition zone diameters of all tested isolates, it can be concluded that the produced T. harzianum cultivation broth shows high efficacy against four maize phytopathogens in vitro. This results once again confirms the great potential of Trichoderma isolates in biological control, which certainly contributes to the development of sustainable agricultural production and consequently has a positive impact on human health.

Key words: *Trichoderma spp., bioprocess, biological control, maize protection, phytopathogenic fungi.*

Introduction

One of the main tasks of modern agricultural production is the development of environmentally friendly methods of plant disease protection, as an alternative to pesticides (Ghazanfar et al., 2018). Consequently, the possibilities of application of biological preparations have recently been increasingly explored and environmentally friendly treatments are also becoming increasingly sought after by both, producers and consumers. Of course, the reason for this is the multiple negative effects caused by synthetic pesticides. Environmental pollution, non-selectivity, the emergence of resistant species, and chemicals in the food chain, has led to necessity of finding new agents with improved characteristics (Lu et al., 2020). Species of the

Tricoderma genus are widely used in the biocontrol of different plant species around the world (Abdel-lateif, 2017; Mukherjee et al., 2014). Large number of *Trichoderma* isolates are located in the ecosystem and wait a moment to be isolated and tested. The great advantage of biocontrol agents is precisely the low cost of their production, high efficiency and practically no effect on human health (Ghazanfar et al., 2018). *Trichoderma* fungi are well known for their antagonism against several soil-phytopathogens, involving fungi, invertebrates, and bacteria (Verma et al., 2007) and *Trichoderma harzianum* is one of the most studied members within this genus (Kuzmanovska et al., 2018). In addition to the remarkable effect in biocontrol, *Trichoderm*a species have a significant impact on plants as growth promoters, but also show important environmental activity, as potential bioremediator (Zin and Badaluddin, 2020). Therefore, in symbiosis with plants, *Trichoderma* can enhance root growth and development, crop productivity, and the use of nutrients, as well as tolerance to abiotic stresses, providing long-term protection for the plants (Lu et al., 2020). Also, *Trichoderma* has found application in industrial production of enzymes, paper and pulp treatment, and food industry (Tripathi et al., 2013).
Maize grain as a rich source of nutrients represents a very good medium for the development of toxic fungi from the genera *Aspergillus, Fusarium* and *Penicillium* (Thompson and Raizada, 2018). The most commonly detected mycotoxins in maize grain are fumonisins, deoxynivalenol, zearalenone and aflatoxins (Krnjaja et al., 2018). Due to a number of serious side effects of these mycotoxins on animals and humans, it is very important to control the presence of phytopathogens on maize. Considering that maize is a significant agricultural crop very present in the human diet, its health safety is of great importance. Accordingly, the aim of this paper is to examine the ability of three *Trichoderma* isolates (*T. harzianum*, *T. citrinoviride* and *T. capillare*) isolated from the environment, in the biological control of the most common maize pathogens, *A. flavus, F. graminearum, H. carbonum* and *Penicillium* sp. This step is very important because not all *Trichoderma* isolates have the same efficacy, and some do not show it at all. Considering that, it is very important to test their performance and select the best for further research.

Materials and Methods

Microorganism antagonist

As microorganisms antagonist, three *Trichodema* isolates: *T. harzianum, T. citrinoviride* and *T. capillare,* were used. Antagonists were isolated from the natural environment and isolates are stored in the Microbial Culture Collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. For inoculum preparation, isolates were initially grown on PDA (Potato Dextrose Agar) for seven days at 25°C. A small amount of mycelium of each isolate was taken for inoculation of 50 ml PDB. The Erlenmayer flakes were incubated for 72 h on a rotary shaker (150 rpm) at 25°C.

Test microorganisms

As a test microorganisms, in the present study phytopathogenic isolates: *A. flavus, F. graminearum, H. carbonum* and *Penicillium* sp., were used. Phytopathogens were isolated from maize plants that showed symptoms of infection (Tančić Živanov et al., 2017). Microorganisms are stored at the PDA medium in the Microbial Culture Collection of Faculty of Technology Novi Sad. Isolates were initially grown on PDA for seven days at 25°C. A small amount of mycelium of each isolate was taken to inoculate 50 ml of PDB. After three days of cultivation, obtained cultivation broth of *A. flavus, F. graminearum, H. carbonum* and *Penicillium* sp. was filtered through double layer of sterile cheesecloth and used for *in vitro* experiment.

Cultivation

The experiment was performed in 100 ml Erlenmeyer flasks with 30 ml of PDB medium and inoculated with a previously prepared inoculum in an amount of 10% relative to the amount of medium. The produced *Trichoderma* cultivation broth was tested after 7 days of cultivation on rotary shaker (150 rpm) at 25°C. In order to obtain the supernatant for *in vitro* analysis, a part of the cultivation broth was centrifuged at 10,000 rpm for 15 minutes (Rotina 380 R, Hettich, Germany) and then further filtered through filter paper. On the other hand, before *in vitro* testing, cultivation broth was passed through a double layer of sterile cheesecloth to separate the layer of formed mycelia.

In vitro technique

In order to compare the activity of three *Tricoderma* isolates on four phytopathogenic fungi, the cultivation broth and the supernatant obtained after 7 days of cultivation under the same, precisely defined conditions, were examined. For *in vitro* test, the well diffusion technique was used (Grahovac et al., 2020). Three wells with a diameter of 15 mm represented one treatment. To each well 100 µl of cultivation broth or supernatant was added. In control plates, 100 µl of sterile distilled water was added to wells. The formed inhibition zone diameters (IZD) were measured after 10 days of incubation at 25 °C.

Data analysis

The obtained data were processed by factorial ANOVA using Software Statistica, version 13.0 (StatSoft Inc., USA). Scheffe multiple range test was used to test significance of differences ($p \leq 0.05$) between mean values of measured diameter of inhibition zones.

Results and Discussion

The results of comparing the activity of the cultivation broth and the supernatant of the three *Tricoderma* isolates showed that the cultivation broth had a significantly better effect on the four tested phytopathogenic isolates compared to the supernatant. This result once again confirms the importance of the activity of *Trichoderma* biomass in the biological control of various plant phytopathogens (Abdel-lateif, 2017). Tables 1 and 3 show the results of factorial analysis of variance for the tested cultivation broth and supernatant of three *Trichoderma* isolates against four phytopathogenic fungi, the causative agents of maize disease.

Table 1. Results of factorial analysis of variance for cultivation broth

Source of variation	SS	Degr. of - Freedom	MS	F-value	p-value
Antagonist	523.56	2	261.78	856.7	0.0000
Test isolate	2578.33	3	859.44	2812.7	0.0000
Antagonist*Isolate	496.67	6	82.78	270.9	0.0000
Error	7.33	24	0.31		

SS – sum of squares; MS – mean square

The results presented in Table 1 show that the aplication of a different antagonist has a statistically very significant effect on the formed inhibition zone ($p<0.01$). This is expected since different *Tricoderma* isolates do not show the same effect on the same phytopathogens (Cherkupally et al., 2017; Dubey et al., 2007). Application of different test isolates shows a statistically very significant influence on the formed inhibition zone diameters, which shows the different sensitivity of tested phytopathogenic isolates to the produced cultivation broths. Also, this influence shows the biggest source of variation. In addition to these influences, the mutual interaction of these two sources of variation also shows a statistically very significant influence on formed inhibition zone diameters. In order to obtain more information about differences in the significance of different *Trichoderma* cultivation broth on test isolates *F. graminearum*, *A. falvus*, *H. carbonum* and *Penicillium* sp., a more detailed post-hoc analysis was performed using Scheffe multiple range test (Table 2).

Table 2. Mean values of IZD (mm) obtained as a result of cultivation broth activity after 10 days of incubation

Antagonist	Test isolate	IZD (mm)
Trichoderma harzianum	*F. graminearum*	50.33 [h]
	H. carbonum	40.67 [f]
	A. flavus	25.67 [d]
	Penicillium sp.	25.33 [cd]
Trichoderma citrinoviride	*F. graminearum*	36.00 [e]
	A. flavus	24.00 [cd]
	Penicillium sp.	23.33 [bc]
	H. carbonum	21.33 [ab]
Trichoderma capillare	*F. graminearum*	45.33 [g]
	H. carbonum	37.33 [e]
	Penicillium sp.	21.00 [a]
	A. flavus	21.00 [a]

*Results are means. The mean values with the same lowercase letters in the column IZD (mm) are not significantly different at 5% level of probability (Scheffe test).

The results from Table 2 show that the best effect on four test phytopathogenic fungi is shown by isolate *T. harzianum*. Since most isolated *T. harzianum* species shows extremely good activity against various phytopathogenic fungi, this result is expected (Mazrou et al., 2020). The obtained inhibition zone diameters formed by the activity of the *T. harzianum* cultivation broth on four test isolates is ordered at the highest level of significance. The broth obtained by cultivation of *T. harzianum* forms the largest inhibition zone diameters, where the largest mean value of the inhibition zone diameter was measured in isolate *F. graminearum* (50.33 mm), then *H. carbonum* (40.67 mm), *A. flavus* (25.67 mm) and the least zone diameter was formed in isolates *Penicilium* sp. (25.33 mm).

Since each inhibition zone larger than 22 mm shows that the produced agent is highly efficient (Tadijan et al., 2016), based on the obtained results for all tested isolates, it can be concluded that the produced *T. harzianum* cultivation broth shows high efficiency against four maize pathogens *in vitro*. According with this fact, the results show that three experiments do not show satisfactory results: *T. citrinoviride* on *H. carbonum* (21.33 mm), *T. capillare* on *A. flavus* (21.00 mm) and *T. capillare* on *Penicillium* sp. (21.00 mm). These experiments are at the lowest level of significance (marked with a lowercase letter *a*).

Table 3. Results of factorial analysis of variance for supernatant

Source of variation	SS	Degr. of - Freedom	MS	F-value	p-value
Antagonist	206.89	2	103.44	169.27	0.0000
Test isolate	468.08	3	156.03	255.32	0.0000
Antagonist*Isolate	132.67	6	22.11	36.18	0.0000
Error	14.67	24	0.61		

SS – sum of squares; MS – mean square

Table 3 represents the results of factorial analysis of variance for the activity of the supernatant obtained by cultivation of three *Trichoderm*a isolates. The activity of the supernatant was tested on four phytopathogenic fungi, causative agents of maize disease. Statistically very significant effect on the formed inhibition zone diameters ($p<0.01$) shows the activity of different antagonists, the sensitivity of different test isolates but also their mutual influence. In order to obtain more detailed information about differences in the significance of the obtained inhibition zone diameters formed by the activity of the supernatant of the three tested *Trichoderma* isolates against four phytopathogenic fungi, detailed post-hoc analysis was performed using the Scheffe multiple range test (Table 4).

Table 4. Mean values of IZD (mm) obtained as a result of supernatant activity after 10 days of incubation

Antagonist	Test isolate	IZD (mm)
Trichoderma harzianum	*F. graminearum*	30.33 [g]
	H. carbonum	26.33 [f]
	A. flavus	18.67 [bc]
	Penicillium sp.	17.67 [abc]
Trichoderma citrinoviride	*F. graminearum*	20.33 [cd]
	A. flavus	17.67 [abc]
	H. carbonum	16.00 [ab]
	Penicillium sp.	15.67 [ab]
Trichoderma capillare	*F. graminearum*	25.00 [ef]
	H. carbonum	23.00 [de]
	A. flavus	16.00 [ab]
	Penicillium sp.	15.00 [a]

*Results are means. The mean values with the same lowercase letters in the column IZD (mm) are not significantly different at 5% level of probability (Scheffe test).

The results presented in Table 4 show that the best activity on *F. graminarum* has a supernatant obtained by cultivating isolate *T. harzianum*. This inhibition zone diameter is at the highest level of significance (marked with a lowercase letter *g*). This is expected considering that other scientists have also confirmed the significant antagonistic effect of *T. harzianum* on different *Fusarium* species (Larran et al., 2020; Cherkupally et al., 2017; Dubey et al., 2007). Supernatant obtained by *T. citrinoviride* does not show the desired activity on this phytopathogenic fungus (zone diameter is less than 22 mm). On the other hand, the best effect on isolate *H. carbonum* shows supernatant *T. harzianum* forming a mean value of the inhibition zone diameter of 26.33 mm. Activity of the produced supernatants of all *Tricoderma* isolates against isolates *A. flavus* and *Penicilium* sp. is unsatisfactory. Figure 1 presents a more detailed comparison of the activity of the produced *Trichoderma* cultivation broth in relation to the supernatant. Figure shows that there is a large difference in the activity of the cultivation broth compared to supernatant for three *Tricoderma* isolates. Also, it can be noticed that the weakest efficiency is shown by the isolate *T. citrinoviride* while the best activity on four phytopathogenic fungi is shown by *T. harzianum*.

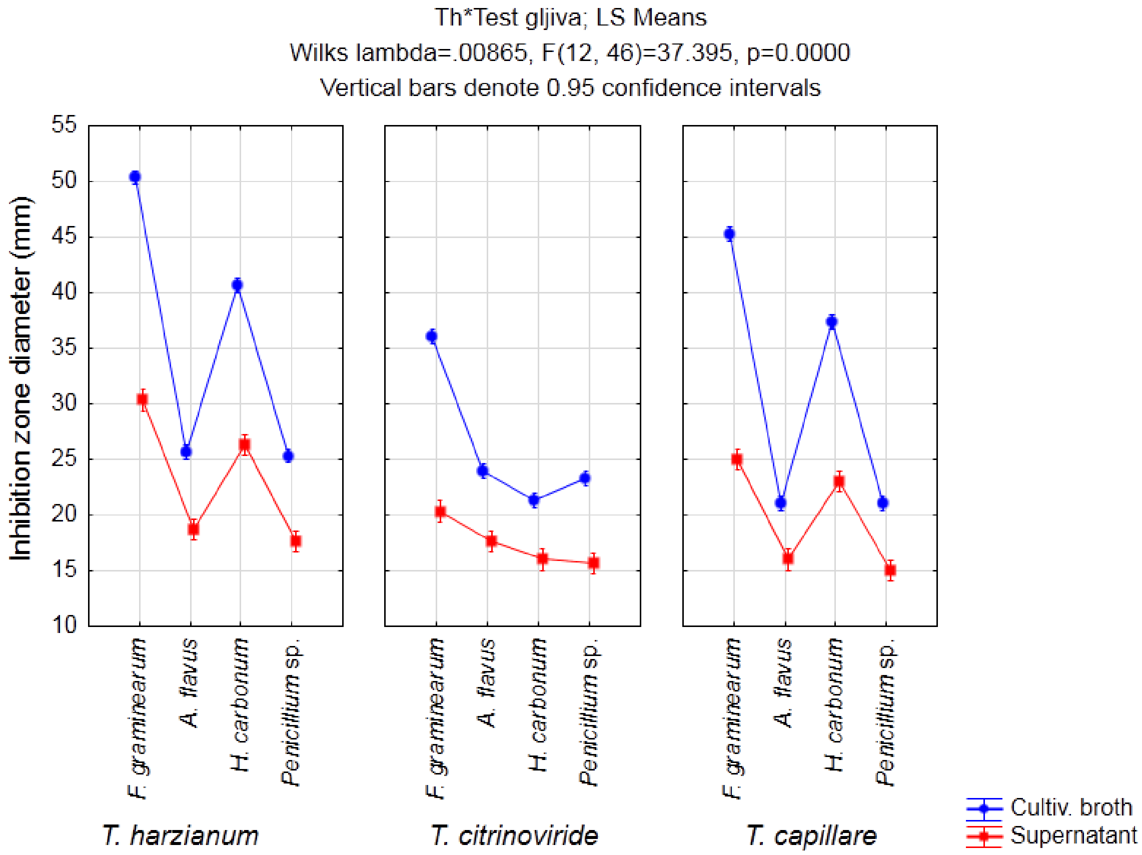

Figure 1. Inhibition zone diameters formed by the activiti of cultivation broth and supernatant obtained by cultivation of three Trichoderma isolates against phytopathogenic fungi: F. graminearum, A. flavus, H. carbonum and Penicillium sp.

For isolate *T. harzianum* it can be noticed that the differences in the activity of the cultivation broth in relation to the supernatant, against four phytopathogenic fungi can be up to 20 mm in the diameter of the inhibition zone. Such a large difference is observed in the activity against fungus *F. graminearum*, which also proved to be the most sensitive. On the other hand, the smallest difference in the activity of the cultivation broth in relation to the supernatant was observed against isolate *A. flavus* and that difference reach value of 7 mm.

Conclusions

The results of this work show that the cultivation broth has a statistically significant efficacy on selected maize pathogens compared to the supernatant, using all *Trichoderma* isolates. By applying the Scheffe test, it was determined that the best effect on the test maize phytopathogens show cultivation broth of *T. harzianum*, forming maximum mean values of inhibition zone diameters of 50.33 mm for *F. graminearum*, 40.67 mm for *H. carbonum*, 25.67 mm for *A. flavus* and 25.33 mm for *Penicillium* sp. This results confirms the great potential of *Trichoderma* isolates, especially *T. harzianum*, in biological control, which certainly contributes to the development of sustainable agricultural production and consequently has a positive impact on human health.

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