Mycotoxin production by entomopathogenic fungus *Conidiobolus coronatus*

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Abstract— *Qualitative and quantitative analysis of selected mycotoxins has been performed in extracts of Conidiobolus coronatus pathogenic fungus cultivated under optimal and stress conditions. Furthermore, the analyses of these compounds in post-incubation filtrates were done. For identification purposes the analytical method allows identification and quantitation of selected mycotoxins including beauvericin , fumonisin B1, enniatin A and B and destruxin A based on high performance liquid chromatography coupled with tandem mass spectrometry was developed. Only beauvericin was detected in very low amounts in C. coronatus mycelium extract cultivated under optimal condition. In the extract of C. coronatus mycelium grown on LB 12.3 ± 0.1 µg/g of beauvericin was determined, while in the extract of C. coronatus mycelium grown on MM medium beauvericin content was lower and amounted 4.6 ± 0.1 µg/g. Also the presence of beauvericin was confirmed in postincubaction filtrate extract (MM). The content of this compound was 2.2 ± 0.1 µg/g. In other extracts beauvericin was not detected. In addition, in the tested extracts other compounds were not detected.*

Keywords— Mycotoxin Production, Conidiobolus, quantitative analysis.

I. INTRODUCTION

The use of chemical insecticides can cause a variety negative effect on the environment. They exhibit high toxicity, but also a low biodegradability and thus accumulate in the environment. As a result of drift by the wind or flushing them torrential rains, these compounds get into reservoirs and waterways. Therefore, it is necessary to search for alternative methods of pest control, which will not have a negative impact on the environment, including humans and animals. The solution to this problem may be use of entomopathogenic fungi. Entomopathogenic fungi are ubiquitous in the environment and plays an important role due to its ability to spontaneous infection reduce the amount of many plant pests [1]. Currently, there are about 3,000 known species of fungi that can cause diseases of living arthropods. Only 30 of them are used as biological agents to limit the number of plant pests [2]. The most of these products is based on the fungi species such as *Metarhizium anisopliae, Beauveria bassiana, Beauveria brongniartii, Paecilomyces fumosoroseus, Lecanicillium longisporum and Lecanicillium muscarium* [3]. These entomopathogenic fungi can be use as bioinsecticides, due to their ability to mass propagation on artificial media [1]. Entomopathogenic fungi produce a number of secondary metabolites which have a different effect on insects [4-7]. *Beauveria bassiana* produces bassianolides - depsipeptide which proved to be important factors in the insect infection [4]. Destruxins produced by the *Metarhizium anisopliae* causes paralysis and death of the infected host [5]. There are also metabolites do not cause the death of organisms, but does not exclude the importance of these compounds in the infection process, e.g. beauverolides not show the insecticidal activity and the immune response [7]. Fumonisins are produced by fungi of the genus *Fusarium*, for example: *F. moniliforme* and *F. proliferatum* occurring primarily in corn grain and its processing products intended for food and feed. The most important analogues found in naturally contaminated corn are fumonisin B1, fumonisin B2 and fumonisin B3. Several strains of fungi *Fusarium* spp produce secondary metabolites belonging to the enniatin group. They are six-membered cyclic depsipeptides having ionophoric, phytotoxic, antiparasitic and antibiotic properties. Beauvericin (BEA) is a toxic metabolite produced by entomopathogenic fungi. This mycotoxin was isolated from an entomopathogenic fungus *Beauveria bassiana* and several other species belonging to the family *Cordycipitaceae* in the *Hypocreales* (*Ascomycota*) [8-11]. *Fusarium* species infecting maize, rice, and wheat are also known as beauvericin producers [9]. There is only one report of BEA occurrence and co-occurrence with fumonisin B1, fumonisin B2 and ochratoxin A. BEA is cyclohexadepsipeptide fungal metabolite with a wide range of biological activities, such as insecticides, anthermintic, antibacterial, antifungal, antiplasmodial, antimycobacterial and anticancer activities. It is the most potent specific inhibitor of cholesterol acyltransferase and possesses ionophoric properties. BEA increases ion permeability in biological membranes by forming a complex with some cations (Ca^{2+}, Na^{+}, K^{+}), which may affect the ionic homeostasis [8]. The insecticidal activity of BEA was first discovered by Hamill et al.[12].

BEA was confirmed as the active compound against *Artimia salina*, which was considered as a model organism to insecticidal activity study. Subsequently, the insecticidal effect of BEA on a microgram level was investigated on *Calliphora erythrocephala, Aedes aegypti, Lygusspp., Spodoptera frugiperda* and *Schizaphis graminum* [13-16]. BEA exhibits toxicity to bacteria: *Bacillus subtiilis, Escherichia coli, Mycobacterium phlei, Sarcinea lutea, Staphylcoccus aureus* and *Streptococcus faecalis* [73]. Furthermore, it is an effective integrase inhibitor of HIV-1 [71]. A very important feature of bEa is the antitumor effect [71]. It interferes with the motility of tumor cells which reduces the speed of many processes in the development of the disease, including the formation of new blood vessels in the tumor cells, and metastasis [53, 77]. It also inhibits the acetyltransferase and cholesterol results in programmed cell death, similar to apoptosis, as well as cytolysis [72, 73, 74].

Conidiobolus coronatus is an opportunistic pathogen with a fairly wide range of infected hosts. For the first time this species was described in 1897 by Costantin'a in France. In contrast, *C. coronatus was* isolated in 1961 by Chester Emmons and Charles Bridges [13, 14]. It occurs commonly in soil and decaying plant material [14]. This entomopathogenic fungus causes the disease process in many arthropods, eg. greater wax moth *(Galleria mellonella)*, pine lappet moth *(Dendrolimus pini)*, springtails and other [13-16]. After penetrating into the body cavity of the fungus kills the insects within 1-2 days, resulting in tissue damage, which is caused by the depletion of nutrients and the production of mycotoxins, which are considered to be the main factor causing the death of an insect. Because of their insecticidal potential *C. coronatus* can be used as a source of new generation bioinsecticides. However, to date, a mycotoxin produced by *C. coronatus* not have been identified and described.

Therefore, the main goal of this study was to assess selected mycotoxins content in extracts of pathogenic fungus *C. coronatus*. As first, different solvents and solvent mixtures were applied to extraction of target analytes. Then, the analytical method based on high performance liquid chromatography coupled with tandem mass spectrometry was developed for qualitative and quantitative determination of these compounds in obtained extracts. The productions of selected mycotoxins by *C. coronatus* grown in rich and poor media were determined. Moreover, the impact of changes in culture conditions, including temperature and pH on mycotoxins production were also assessed. The results of this study provide useful information to assess the potential use of *C. coronatus* as a source of new generation bioinsecticides.

II. EXPERIMENTAL PART

2.1 Chemicals

Beauvericin, fumonisin, enniatin A and B, destruxin were obtained from Sigma Aldrich. HPLC-grade acetonitrile and methanol, dichloromethane, hexane, ammonium acetate, acetic acid was purchased from POCh. Stock solutions containing all tested mycotoxins were prepared at concentration 50 µg/mL, working solution were prepared by appropriate dilution of stock solution.

2.2 Conidiobolus coronatus

The subject of the studies was the entomopathogenic fungus *C. coronatus* belonging to the class of *Zygomycota* and the order of *Entomophthorales*. Mycelium was obtained from cultures of the insecticidal fungus *C. coronatus* grown in 30 L of liquid minimal medium (MM pH 7.0, consisting of 0.1 % of $(NH_4)_2SO_4$, 0.45 % of KH_2PO_4, 1.05 % of K_2HPO_4, 0.05% of sodium citrate dehydrate, 0.2 % of glucose and 0.025 % of MgSO4 all as mass %) and liquid nutrient-rich medium (LB consisting of 1 % of tryptone, 0.5 % of yeast extract, 0.5 % of NaCl and 0.1 % of 1 M NaOH)) in optimal and stress condition. After 3 weeks, the mycelium was filtered on filter papers, collected and stored at -20^{o}C.

2.3 Optimization of extraction conditions

To 1 g of finely ground sample 50 µl of a mixture solution containing target analytes in MEOH at concentration 5 µg/ml were spread over the surface. The flask was shaken manually to distribute the added standards as evenly as possible. The sample was left open at room temperature for 2 h. Three extraction solvent mixtures such as: methanol-water (70:30, v/v), ACN: MeOH: H_2O (16: 3: 1, v/v); and ethyl acetate were used. After 12 h, the extract was filtered through the filter paper. The extraction procedure was repeated once again. Then 25 ml of hexane was added and the mixture was shaken for 10 min. The hexane layer was discarded, and the remainder layer was concentrated to dryness on a rotary evaporator. The residue was dissolved in a mixture of MeOH: H_2O (1: 1, v / v) at a volume of 15 ml and extracted twice with 7.5 ml of dichloromethane. The layers of dichloromethane were combined, then evaporated to dryness using a rotary rotator. The

residue was then dissolved in 1 ml of ACN and transferred to vials. As described above, the native sample of the *C. coronatus* mycelium was also prepared. All extracts were analyzed by LC-MS/MS.

2.4 Extraction of *C. coronatus* mycelium grown in optimal and stress conditions

A samples of *C. coronatus* mycelium grown in optimal and stress conditions were lyophilized. To the resulting lyophilisate 20 ml of a mixture ACN: MeOH: H_2O (16: 3: 1, v / v) was added and left for 12 h. After that, the extract was filtered and extracted once again using 20 ml of solvent mixture mentioned above (12 h). Then 25 ml of hexane was added and mixture was shaken for 10 min. The hexane layer was discarded, and the remainder layer was concentrated to dryness on a rotating rotor. The residue was dissolved in 15 ml of MeOH: H_2O (1:1, v/v) and extracted twice with 7.5 ml dichloromethane. The layers of dichloromethane were combined, evaporated to dryness. The residue was then dissolved in 1 ml of ACN and analyzed by LC-MS/MS. Table 1 summarizes the culture conditions and the weight of the extracts of *C. coronatus* mycelium nad post-incubation filtrates.

TABLE 1
***C. CORONATUS* MYCELIUM MASSES BEFORE AND AFTER EXTRACTION BY ACN: MeOH: H_2O (16:3:1, v/v/v)**

The culture conditions	Masses of *C. coronatus* mycelium [g]	Masses of extracts [mg]
MM medium, optimal condition	6.91	50.9
LB medium, optimal condition	4.16	25.7
MM medium, pH 5, temperature 30 °C	1.49	12.6
MM medium, pH optimal, temperature 30 °C	1.87	18.9
MM medium, pH 5, temperature 20 °C	1.29	22.9
MM medium, pH optimal, temperature 20 °C	1.09	17.9
LB medium, pH 5, temperature 30 °C	1.51	11.9
LB medium, pH optimal, temperature 30 °C	0.99	10.8
LB medium, pH 5, temperature 20 °C	1.04	15.7
LB medium, pH optimal, temperature 20 °C	2.9	14.1
Post-incubation filtrate (MM)	100 ml	89.7
Post-incubation filtrate (LB)	100 ml	124.7

2.5 Extraction of post-incubation filtrates

Post-incubation filtrates obtained after *C. coronatus* culture on MM and LB medium were lyophilized and weighted. Then 75 ml of a mixture ACN: MeOH: H_2O (16: 3: 1, v / v) was added and samples left for 12 h. At this time extracts were filtered and extraction was repeated with a mixture of solvents for 12 h . After this time extracts were filtered, combined, and shaken with 25 mL of hexane for 10 min. The hexane layers were discarded, whereas the layer of ACN: MeOH: H_2O was concentrated to dryness using a rotary rotator. The resulting residue was dissolved in a mixture of MeOH:H_2O (15 ml, 1:1, v/v) and extracted twice with 7.5 ml dichloromethane. The dichloromethane layers were combined, and then evaporated to dryness using a rotary rotator. Thus prepared extracts were dissolved in 1 ml of ACN and analyzed by LC-MS/MS.

2.6 LC-MS analysis

Chromatographic analysis was performed using liquid chromatography Series 1200 (Agilent Technologies) with Ultra HCT mass spectrometer (Brucker Daltonics) equipped with a column Hypersil Gold a Q C18 (150×4,6 mm, 5 µm). As the mobile phases: ACN (phase A), 1 mM aqueous solution CH_3COONH_4 (phase B): ACN (90:10, v / v) at pH 3.5 were used. The separation was carried out under gradient elution conditions from 10% B to 90% phase B (20 min.) and then 90% of phase B by 5 minutes. The following mass spectrometer parameters were applied: drying gas – nitrogen, drying the gas pressure - 10 psi, drying gas flow rate - 7 l/min., drying gas temperature - 300 ° C, capillary voltage - 4 kV.

III. RESULTS AND DISCUSSION

Quantification of mycotoxin is usually carried out by liquid chromatography coupled with mass spectrometry (LC-MS(/MS)) or gas spectrometry coupled with mass spectrometry often within a multi-analytes approach without any clean-up procedure. For accurate quantification, liquid chromatography equipped with UV-Vis detector can be also applied because of using these techniques allows to avoid of matrix effects. None of the applied methods has been formally evaluated in inter-laboratory validation studies and there are no certified reference materials available for beauvericin determination in natural samples. In our work the LC-MS method for determination of beauvericin in mycelium and post-incubation filtrates was developed. The optimum parameters of the mass spectrometer and a liquid chromatography allowing the isolation and detection of all analytes, ie.: beauvericin, destruxin A, fumonisin, enniatin A and B were selected. Optimization of the mass spectrometer was done in full scan range of *m/z* 50-1000 Da. Analysis of mass spectra obtained in positive ion mode for beauvericin shows the presence of the ion $[M + Na]^+$ *m/z* 806.7. In addition, ions $[M+NH_4]^+$ at *m/z* 801.7 and $[M+K]^+$ at *m/z* of 822.7 were also present. In the mass spectrum recorded in negative ion mode, the signal with the highest intensity corresponds to the ion $[M-H]^-$ at *m/z* 782.6. The mass spectrum recorded in the positive ion mode for destruxin A presence of a signal of *m/z* 600.6 which corresponds to $[M + Na]^+$ ion was detected. Furthermore, in this mass spectrum the ions: $[M + H]^+$ at *m/z* 578.6 and $[M + K]^+$ at *m/z* 617.6 were also denoted. As a result of negative ionization of the analyte a signal for *m/z* corresponding to the ion $[M-H]^-$ was not present. For compounds from the group enniatin (enniatin A and B) both positive and negative ionization mode gave signals of pseudomolecular ions. in the negative ion mode signals at *m/z* 680.6 and 638.6 were present whilst in the positive ionization mode the presence of ions $[M + Na]^+$, $[M + NH4]^+$ and $[M + H]^+$ was observed. Analysis of mass spectra obtained in positive ion mode for fumonisin B1 shows the presence of the $[M + H]^+$ ion at *m/z* 722.7. Moreover, the ions: $[M + Na]^+$ at *m/z* and 744.7 $[M + K]^+$ at *m/z* 760.6 were detected. In the spectrum registered in negative ion mode, a signal corresponding to $[M-H]^-$ at *m/z* 720.6 was observed. In the next stage of the research the conditions of chromatographic separation of analyzed compounds was studied. In the application of available software, complete separation of the chromatographic signals is not necessary however; their separation can significantly improve the sensitivity and selectivity of developed method. Furthermore, the retention time is an additional parameter to permit identification of analytes in real samples. The use of gradient elution from 10% to 90% phase B, then 90% of phase B for 5 minutes allowing obtaining optimum conditions in terms of the retention time, separation of the compounds and symmetry of signals. Pseudo-molecular and fragmentation ions selected for qualitative analysis were summarized in Table 2. Validation parameters of the LC-MS method were summarized in Table 3.

TABLE 2
SELECTED PSEUDO-MOLECULAR AND FRAGMENTATION IONS FOR MYCOTOXINS DETERMINATION

Mycotoxin	Retention time [min.]	Pseudomolecular ion		Fragmentation ions
Fumonisin B1	16.8	$[M+H]^+$	*m/z* 723	352.4 528.6 704.7
Destruxin A	19.8	$[M+H]^+$	*m/z* 579	178.1 437.4 465.5 550.5
Enniatin B	30.8	$[M+NH_4]^+$	*m/z* 658	196.3 640.7
Beauvericin	32.4	$[M+NH_4]^+$	*m/z* 802	244.2 262.3 542.5 784.7
Enniatin A	33.9	$[M+NH_4]^+$	*m/z* 700	210.3 455.4 682.7

TABLE 3
METHOD VALIDATION PARAMETERS OF THE FINAL MYCOTOXINS DETERMINATION BY LC-MS/MS

Mycotoxin	Coefficient of determination R^2	Accuracy [%]	Precision [%]	IQL [µg/ml]	IDL [µg/ml]
Beauvericin	0.9998	103.7 – 109.1	1.47 – 5.32	0.06	0.02
Enniatin A	0.9998	101.26 –113.21	1.83 –2.97	0.06	0.02
Enniatin B	0,9992	97.92 –103.75	1.53 –4.03	0.06	0.02
Fumonisin B1	0,9982	94.33 –101.43	2.38 –5.73	0.06	0.02
Destruxin A	0.9964	92.32 –106.64	0.88 –2.85	0.06	0.02

3.1 Identification and quantitation of selected mycotoxin in extracts of *C. coronatus* mycelium and post-incubation filtrates

Fungi in *Entomophthorales*, including *C. coronatus*, are subject to interest of researchers due to the possibility of their use in biological pest control. This is related to relatively short infection cycle, high index of reproduction and the ability to induce epizootic in ecosystems. Most of them are specialized in infecting a specific group of hosts, so that does not pose a risk to non-target organisms. Mycotoxins produced by entomopathogenic fungi have insecticidal properties, and preparations containing the fungal spores capable of produce them are now increasingly being used in biological pest control. In addition, this kind of insecticides is an alternative to chemical compounds, the use of which in excess is hazardous to the environment and living organisms. This is the first report of the detection of beauvericin in extracts of *C. coronatus*. Several extracts of *C. coronatus* were analysed and the impact of the grown conditions on beauvericin production were assessed. The isolation of mycotoxins from *C. coronatus* mycelium and post-incubation filtrates was done by solvent extraction with different methods. As first ethyl acetate was used due to the fact, that it is one from four most commonly used solvent for the extraction of mycotoxins from biological samples. Extraction by mixture of solvents ACN: MeOH: H_2O (16: 3: 1, v / v) and MeOH: H_2O (7:3, v/v) was also applied to mycotoxin isolation from *C. coronatus*. As was shown in Table 4, the extraction efficiency of target analytes were the highest using ACN: MeOH: H_2O (16: 3: 1, v / v). The recoveries of selected mycotoxin were in the range from 63.5% to 75.5 %. Therefore this mixture was applied as a extraction solvent to isolation mycotoxins produced by *C. coronatus*.

TABLE 4
PERCENTAGE OF RECOVERY FOR SELECTED MYCOTOXINS IN SPIKED MYCELIUM OF *C. CORONATUS* BY USING DIFFERENT EXTRACTION SOLVENTS

Mycotoxin	Recovery [%]		
	Ethyl acetate	ACN: MeOH: H_2O (16:3:1, v/v/v)	MeOH: H_2O (7:3, v/v)
Fumonisin B1	46.4	63.5	73.4
Destruxin A	43.7	67.8	62.3
Enniatin B	55.3	72.4	58.4
Beauvericin	53.8	75.3	67.4
Enniatin A	58.5	69.4	54.8

The results of LC-MS analysis indicated the presence of beauvericin in the extract mycelium of *C. coronatus* and the post-incubation filtrates. In the mass spectrum obtained in the LC-MS analysis of the extract of the *C. coronatus* mycelium, the molecular peak at m/z 802 and a signals at m/z 244.2; 262.3; 541.5; 542.6; 784.6; 811.4 confirmed the trimeric structure of beauvericin and clearly indicates the presence of this compound in analyzed extracts. Results of beauvericin production by *C. coronatus* isolates are summarized in Table 5. In the case of extract of C. coronatus mycelium grown on LB medium was 11.8 ± 0.1 µg/g, while grown on MM medium - 5.2 ± 0.3 µg/g. Also the presence of beauvericin was confirmed in post-incubaction filtrate extract (MM). The content of this compound was 2.2 ± 0.1µg/g. In extracts of *C. coronatus* mycelium

grown under conditions other than the optimal beauvericin content was detected in extracts of *C. coronatus* mycelium grown on both LB and MM medium, at temperature 20 °C and optimal pH condition. In the extract of *C. coronatus* mycelium (MM medium 20, pH optimum) the presence of 1.5 ± 0.1 µg/g beauvericin was confirmed. While in mycelium of *C. coronatus* grown on LB medium (20, optimal pH) beauvericin content was 4.9 ± 0.1 µg/g in the extract. The highest BEA yield was detected in extract of *C. coronatus* mycelium cultures grown in LB medium in optimal condition. Three times lower contents of BEA was determined in the extract of *C. coronatus* mycelium grown on MM medium under optimal pH and temperature conditions. The lower content of BEA mainly attributed to the type of medium which contains only those components that are essential to sustain vital functions. The effect of changes in the pH of the culture medium on BEA content in *C. coronatus* mycelium was also observed. Temperature changes also affect the BEA content in the extracts, it was observed that in the case of temperature reduction the contents of the BEA is lower than in optimal conditions, while with an increase of temperature BEA was not detected. Beauvericin was originally identified in entomopathogenic fungi, such as *Beauveria bassiana* and *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*)[9]. As the main beauvericin producers and the species responsible for its accumulation *F. subglutinans* and *F. proliferatum* were identified. Previous studies reported the natural occurrence of beauvericin in maize infected by entomopathogenic fungi [17-21].

TABLE 5
MYCOTOXIN CONCENTRATION FOUND IN HOMOGENATES AND POST-INCUBATION FILTRATES OF *C. CORONATUS* CULTIVATED AT DIFFERENT CONDITIONS

The culture conditions	Fumonisin [µg/g]	Beauvericin [µg/g]	Destruxin [µg/g]	Enniatin A and B [µg/g]
MM medium, optimal condition	nd	5.2 ± 0.3	nd	nd
LB medium, optimal condition	nd	11.8 ± 0.1	nd	nd
MM medium, pH 5, temperature 30 °C	nd	nd	nd	nd
MM medium, pH optimal, temperature 30 °C	nd	nd	nd	nd
MM medium, pH 5, temperature 20 °C	nd	nd	nd	nd
MM medium, pH optimal, temperature 20 °C	nd	1.5 ± 0.1	nd	nd
LB medium, pH 5, temperature 30 °C	nd	nd	nd	nd
LB medium, pH optimal, temperature 30 °C	nd	nd	nd	nd
LB medium, pH 5, temperature 20 °C	nd	nd	nd	nd
LB medium, pH optimal, temperature 20 °C	nd	4.9 ± 0.1	nd	nd
Post-incubation filtrate (MM)	nd	2.2 ± 0.1	nd	nd
Post-incubation filtrate (LB)	nd	nd	nd	nd

The results of qualitative and quantitative analysis of the BEA in five mycelium samples of different strains of *Fusarium subglutinans* and in corn samples infected by this species are described in the literature [16]. *F. subglutinans* strains produced BEA at a level of 140 mg/kg to 700 mg/kg, while in the maize samples revealed the presence of the BEA content from 7.6 to 238.8 µg/kg. BEA production capacity by various *Fusarium* strains were also described by A. Logrieco et al.[9]. Among the 94 strains belonging to 25 different species of the *Fusarium*, the ability to beauvericin production revealed: *F. sambucinum, F. longipes, F. subglutinans, F. anthophilum, F. oxysporum, F. poae, F. avenaceum, F. beomiforme, F. dlamini, F. nygamai*. The highest beauvericin producer was one of the two tested strains of *F. longipes* Wollenw. et Reinking (ITEM-3202) (200 µg/g). Other beauvericin-producing species were *Fusarium acuminatum* Ell. et Ev. var. acuminatum (one of four isolates), *Fusarium acuminatum* var. armeniacum Forbes et al. (one of three isolates), and F. equiseti (Corda) Sacc. (two of three isolates). In the *Sporotrichiella* section, all four tested strains of *F. poae* (Peck) Wollenw. Produced the toxin, ranging from traces to 63 µg/g.

In the literature, there is little information about the effects of external factors on the mycotoxins production by entomopathogenic fungi. According to Hodgson (2000) optimal temperature for the production of mycotoxins by *F. langsethiae and F. sporotrichioides* is lower than a temperature that is optimal for their growth [22]. In 2006 the effects of temperature on the effectiveness of fungal pathogens in reducing the number of different developmental stages of *Frankliniella occidentalis* were carried out. The aim of study was to determine the effect of temperature: 20 °C, 25 °C and 30 °C on the effectiveness of *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii* and *Paecilomyces lilacinus*. After the experiment, it was found that the temperature has an effect to reduce the number of the western flower thrips by tested entomopathogenic fungi. The obtained results shown that *P. lilacinus* is effective pathogen for western flower thrips reduction in all the temperatures and their effectiveness was in the range of 68% to 76%. *M. anisopliae* was the most

effective at 25 ° C, and its efficiency was 82%. An increase or decrease in temperature caused a decrease in efficiency of this fungus. Similarly was in case of *B. bassiana*. The fungus *L. lecanii* was more effective in reducing of western flower thrips population with increasing temperatures, for example, at 30 °C caused 84% mortality of larval stages, but in relation to the adult insects its effectiveness at 30 °C does not exceed 50%. The highest mortality of western flower thrips, i.e. 92% was noted by the pathogens *B. bassiana* and *M. anisopliae* at 25 °C [23].

The results of LC-MS analysis revealed no ions indicating the presence of other compounds, including enniatin A and B, destruxin A, fumonisin B1. Therefore, it can be concluded that in extracts of mycelium and post-incubation filtrates of *C. coronatus* the above-mentioned compounds have not been present.

IV. CONCLUSIONS

Economic losses caused by harmful insects are a serious economic problem. Reducing of the pests are still made mainly using non-selective chemical insecticides, the accumulation of which in the environment is a serious threat to biodiversity and human health. Accompanying this process the growing resistance of harmful insects to classical insecticides, tends to reduce the use of chemical insecticides and the search for new, selective and less environmentally harmful methods of pest control. In this work the isolation and qualitative and quantitative analysis of mycotoxins in the mycelium and post-incubation filtrates of the *C. coronatus* using chromatographic methods were done. In the extracts C. coronatus mycelium grown in LB and MM medium the presence of BEA was confirmed (12.3 ± 0.1 µg/g and 4.6 ± 0.1 µg/g, respectively). In turn, the content of BEA in the post-incubation filtrate (MM) was 2.2 ± 0.1 µg/g. On the other hand, in the test samples of mycelium and post-incubation filtrates the presence of compounds from the group of enniatin, fumonisins and destruxin were not determined.

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REFERENCES

[1] Tomalak M., Biological agents available in organic crop protection against pests, Institute of Plant Protection - National Research Institute in Poznan, 2009, 13-15

[2] Malinowski H., Ability to protect the forest from insects damaging root systems biological method using entomopathogenic fungi. The mechanism of infection of insect and fungus entomopathogenic factors infection, Sylwan, 2009, **153** (12), 795−804

[3] Charnley A.K., Collins S.A., Entomopathogenic fungi and their role in pest control, Environmental and Microbial Relationships,The Mycota IV, 2007, R.10, 159-187

[4] Glare T. R., Goettel M.S., Eilenberg J., Addendum: Entomopathogenic fungi and their role in regulation of insect populations 2004-2009, Insect control, Academic Press, 2010, 433-437

[5] Molnár I., Gibson D.M., Krasnoff S.B., Secondary metabolites from entomopathogenic Hypocrealean fungi, Natural Product Reports, 2010, **27** (9), 1233–1372

[6] Piątkowski J., Krzyżewska A., Nawrot U., Przeciwgrzybicza akywność entomopatogenicznego szczepu *Paecilomyces* sp., Mikologia lekarska, 2003, **10** (2), 93-99

[7] Kuzma M., Jegorov A., Kačer P., Havlíček V., Sequencing of new beauverolides by high-performance liquid chromatography and mass spectrometry, Journal Of Mass Spectrometry, 2001, **36**, 1108–1115

[8] Wang Q., Xu L., Beauvericin, a bioactive compound produced by fungi: a short review, molecules, 2012, **17**, 2367-2377

[9] Logrieco A., Moretti A., Castella G., Kostecki M., Golinski P., Ritieni A., Chelkowski J., Beauvericin Production by *Fusarium* Species, Applied and Environmental Microbiology, 1998, **64** (8), 3084-3088

[10] Vey A., Hoagland R.E., Butt T.M., Toxic metabolites of fungal biocontrol agents, Fungi as Biocontrol Agents, Progress, Problems and Potential, Cabi, 2001, R.12, 311-346

[11] Buscano Flor L., Interaction of an Entomopathogenic Fungus and Black-legged Ticks, *Ixodes Scapularis* (Say) (Acari: Ixodidae), University of Minnesota, Praca doktorska, 2006

[12] Hamill, R. L., C. E. Higgens, H. E. Boaz, and M. Gorman.1969. The structure of beauvericin: a new depsipeptide antibiotic toxic to Artemia salina. Tetrahedron Lett. 49:4255–4258.n

[13] Barta M., Fungi of the order *Entomophthorales* infecting aphids in Slovakia, Slovak University of Agriculture, Praca doktorska, 2004

[14] http://mushroomobserver.org/name/show_name_description/1856?_js=on&_new= true, dostępna 22. 04.13 r.

[15] Ellis D., Davis S., Alexiou H., Handke R., Bartley R., Descriptions of medical fungi, School of molecular & biomedical science, University of Adelaide, 2007

[16] Humber R.A., Entomopathogenic fungal identification, USDA-ARS Plant Protection Research Unit, US Plant, Soil & Nutrition Laboratory, 2005

[17] Ritieni A, Moretti A, Logrieco A, Bottalico A, Randazzo G, Monti S M, Ferracane R, Fogliano V. Occurrence of fusaproliferin, fumonisin B1, and beauvericin in maize from Italy. J Agric Food Chem. 1997;45:4011–4016.

[18] Moretti A, Logrieco A, Bottalico A, Ritieni A, Randazzo G, Corda P. Beauvericin production by Fusarium subglutinans from different geographical areas. Mycol Res. 1995;99:282–286.

[19] Moretti A, Logrieco A, Bottalico A, Ritieni A, Fogliano V, Randazzo G. Diversity in beauvericin and fusaproliferin production by different populations of Gibberella fujikuroi (Fusarium section Liseola) Sydowia. 1997;48:44–56.

[20] Plattner R D, Nelson P E. Production of beauvericin by a strain of *F. proliferatum* isolated from corn fodder for swine. Appl Environ Microbiol. 1994;60:3894–3896.

[21] Hamill R L, Higgens C E, Boaz H E, Gorman M. The structure of beauvericin: a new depsipeptide antibiotic toxic to *Artemia salina*. Tetrahedron Lett. 1969;49:4255–4258.

[22] Hodgson, D.A., 2000. Primary metabolism and its control in streptomycetes: a most unusual group of bacteria, Adv. Microb. Physiol. 42, 47-238.

[23] Fiedler Ż., Sosnowska D. (2006) Wpływ temperatury na efektywność patogenów grzybowych w ograniczaniu liczebności różnych stadiów rozwojowych Frankliniella Occidentalis (Pergande), Progress in Plant Protection / Postępy w Ochronie Roślin 46 (4).