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Stacjonarne Studia Doktoranckie Mikrobiologii, Biotechnologii i Biologii Eksperymentalnej

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Mechanizmy oporności grzybów entomopatogennych na insektycydy z grupy pyretroidów

Mechanisms of resistance of entomopathogenic fungi to insecticides from the group of pyrethroids

Praca doktorska
wykonana w Katedrze Mikrobiologii Przemysłowej i Biotechnologii, Instytutu Mikrobiologii, Biotechnologii i Immunologii

pod kierunkiem
dr hab. Sylwii Różalskiej, prof. UŁ

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Pragnę złożyć najserdeczniejsze podziękowania mojej Pani Promotor -
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że byłam w stanie zdobyć nie tylko cele naukowe, lecz także podjąć wyzwania,
które postawiło przede mną życie. Nie jestem w stanie wyrazić słowami
wdzięczności, jaką czuję.

Lista używanych skrótów

AAS (Atomic Absorption Spectrometry, Absorpcyjna spektrometria atomowa); **ARSEF** (Agricultural Research Service Collection of Entomopathogenic Fungal Cultures); **ATP** (adenosine triphosphate, adenozynotrifosforan); **CAT** (catalase, katalaza); **DAG** (diacylglycerol, diacyloglicerol); **ETC** (Electron Transport Chain, Łańcuch Transportu Elektronów,) **GABA** (gamma-aminobutyric acid, kwas gamma-aminomasłowy); **GC-MS** (Gas Chromatography with tandem mass spectrometry, Chromatografia gazowa sprzężona z tandemową spektrometrią mas); **GPx** (glutathione peroxidase, peroksydaza glutationowa); **GST** (glutathione S-transferase, S-transferaza glutationowa); **HSP** (Heat Shock Protein, białka szoku cieplnego); **H_2O_2** (hydrogen peroxide, nadtlenek wodoru); **NO** (nitric oxide, tlenek azotu); **O_2•-** (superoxide anion radical, anionorodnik ponadtlenkowy); **PA** (phosphatidic acid, kwas fosfatydowy); **PC** (phosphatidylcholine, fosfatydylocholina); **PE** (phosphatidylethanolamine, fosfatydyloetanoloamina); **PI** (phosphatidylinositol, fosfatydyloinozytol); **PR1** (serine endoprotease, endoproteaza serynowa); **PR2** (trypsin-like acid protease, kwaśna proteaza trypsynopodobna); **PS** (phosphatidylserine, fosfatydyloseryna); **RFT** (reaktywne formy tlenu (ang. reactive oxygen species - **ROS**)); **SOD** (superoxide dismutase, dysmutaza ponadtlenkowa); **TAG** (triacylglycerol, triacyloglicerol); **2,4-D** (2,4-dichlorophenoxyacetic acid, kwas 2,4-dichlorofenoksyoctowy).

Spis treści

Źródła finansowania badań prowadzonych w ramach rozprawy doktorskiej

1. **Grant NCN 2016/23/B/NZ9/00840**: „Określenie potencjału infekcyjnego grzybów entomopatogennych w obecności insektycydów chemicznych" (2016-2023)
   Kierownik projektu: **Dr hab. Sylwia Różalska, prof. UŁ**
2. Dotacja celowa na działalność związaną z prowadzeniem badań naukowych lub prac rozwojowych oraz zadań z nimi związanych, służących rozwojowi młodych naukowców oraz uczestników studiów doktoranckich pt. „Oznaczenie markerów stresu oksydacyjnego u grzybów entomopatogennych traktowanych insektycydami z grupy pyretroidów".
   Kod projektu: B1811000001838.02
3. Dotacja celowa na działalność związaną z prowadzeniem badań naukowych lub prac rozwojowych oraz zadań z nimi związanych, służących rozwojowi młodych naukowców oraz uczestników studiów doktoranckich pt. „Określenie wpływu insektycydów z grupy pyretroidów na grzyby entomopatogenne".
   Kod projektu: B1911000002156.02

NARODOWE CENTRUM NAUKI

Ministerstwo Nauki i Szkolnictwa Wyższego

Publikacje wchodzące w skład rozprawy doktorskiej

P1 - Litwin A., Nowak M., Różalska S. February 2020. Entomopathogenic fungi: unconventional applications. *Reviews in Environmental Science and Bio/Technology*, vol. 19, 23-42.
https://doi.org/10.1007/s11157-020-09525-1

IF_{2021} = 14.284; $IF_{5\text{-letni}}$ = 12.751; punkty MEiN = 140; 48 cytowań

P2 - Litwin A., Bernat P., Nowak M., Słaba M., Różalska S. October 2021. Lipidomic response of the entomopathogenic fungus *Beauveria bassiana* to pyrethroids. *Scientific Reports*, vol. 11, 21319.
https://doi.org/10.1038/s41598-021-00702-y

IF_{2021} = 4.997; $IF_{5\text{-letni}}$ = 5.516; punkty MEiN = 140; 3 cytowania

P3 - Litwin A., Mironenka J., Bernat P., Soboń A., Różalska S. January 2023. Accumulation of pyrethroids induces changes in metabolism of the entomopathogenic fungus *Beauveria bassiana*—Proteomic and lipidomic background. *Ecotoxicology and Environmental Safety*, vol. 249, 114418.
https://doi.org/10.1016/j.ecoenv.2022.114418

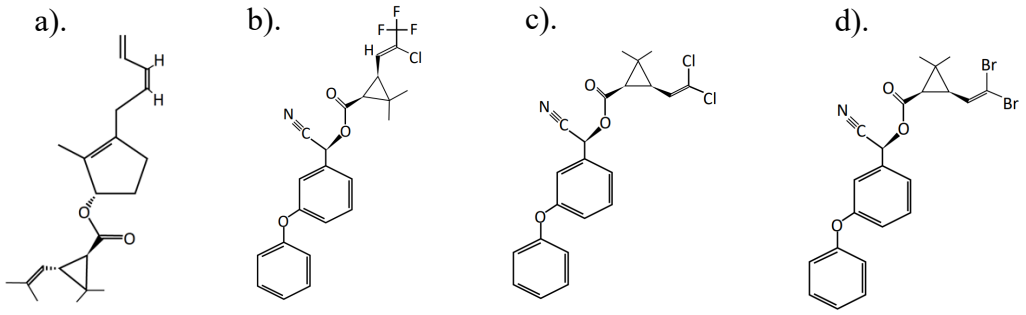
IF_{2021} = 7.129; $IF_{5\text{-letni}}$ = 7.284; punkty MEiN = 100; brak cytowań

Sumaryczny IF_{2021} = 26.41
Sumaryczny $IF_{5\text{-letni}}$ = 25.551
Łączna liczba punktów MEiN = 380

Podane wartości IF, punktacja MEiN oraz liczba cytowań są zgodne z listą obowiązującą w 2023 roku.
Wartości IF – baza Journal Citation Reports™, stan na dzień 26.01.2023 r.
Liczba cytowań – baza Web of Science, stan na dzień 26.01.2023 r.

I. Wprowadzenie

Zaawansowany i postępujący rozwój przemysłu agrochemicznego odgrywa znaczącą rolę w ochronie roślin przed szkodnikami niszczącymi uprawy rolne. Powszechne stosowanie środków ochrony roślin umożliwia zwiększenie plonowania, ale prowadzi także do nagromadzenia pozostałości tych substancji w środowisku naturalnym, co niesie negatywne skutki dla organizmów żywych (Chrustek i in. 2018; Tang i in. 2021). Na szczególną uwagę zasługują insektycydy, czyli środki stosowane do zwalczania owadów. Wśród dostępnych na rynku syntetycznych insektycydów znajdują się związki, które różnią się nie tylko budową i właściwościami chemicznymi, ale także toksycznością oraz przeznaczeniem ich wykorzystania. Do insektycydów zaliczamy: neonikotynoidy, karbaminiany, insektycydy chloro- i fosforoorganiczne, ryanoidy oraz pyretroidy. Pyretroidy, na których skupia się niniejsza rozprawa, są stosowane rutynowo (Zhang i in. 2021). Jak podają Li i in. (2017), pyretroidy są trzecią najczęściej stosowaną grupą insektycydów na świecie. Te syntetyczne insektycydy pod względem chemicznym są analogami pyretryn (Ryc. 1). Mechanizm działania tych substancji polega na zaburzaniu funkcjonowania układu nerwowego, poprzez zwiększanie przewodzenia impulsów, prowadzące do uszkodzenia neuronów, interakcje z kanałami sodowymi bramkowanymi napięciem oraz kanałami chlorkowymi bramkowanymi kwasem gamma-aminomasłowym (GABA). Interakcje te prowadzą do opóźniania aktywacji kanałów sodowych i przedwczesnego ich otwierania oraz hamowania działania kanałów chlorkowych (Bhatt i in. 2019).



Ryc.1. Wzory strukturalne pyretroidów: pyretryny I (a), λ-cyhalotryny (b), α-cypermetryny (c) oraz deltametryny (d).

Od ponad 30 lat pyretroidy są szeroko stosowane do zwalczania stawonogów zarówno w rolnictwie, leśnictwie, jak również w budynkach użyteczności publicznej, szklarniach oraz domach mieszkalnych (Cycoń i Piotrowska-Seget 2016; Tang i in. 2018). Powszechne

stosowanie pyretroidów oraz ich lipofilność i hydrofobowość prowadzą do nagromadzenia ich pozostałości w glebie, wodach powierzchniowych i osadach (Cycoń i Piotrowska-Seget 2016; Tang i in. 2018). Ponadto, substancje te z łatwością przedostają się z gleby do wód powierzchniowych z którymi są transportowane na duże odległości do rzek i źródeł wody pitnej, co przyczynia się do wzrostu potencjalnego zagrożenia dla zdrowia ludzi (Tang i in. 2021).

Pomimo, że pyretroidy są uznawane za mało toksyczne w porównaniu do insektycydów z innych grup, wykazano, że narażenie ludzi na te substancje prowadzi do zaburzeń funkcjonowania układu nerwowego, immunologicznego i hormonalnego (Chrustek i in. 2018; Tang i in. 2021). Do organizmu ludzkiego przedostają się głównie przez skórę, drogi oddechowe i układ pokarmowy (Chrustek i in. 2018). Wykazano również, że pyretroidy są silnie szkodliwe dla organizmów wodnych i glebowych (Cycoń i Piotrowska-Seget 2016; Tang i in. 2018). W związku ze szkodliwym wpływem pyretroidów, wzrasta zainteresowanie tematem zanieczyszczenia środowiska tymi substancjami (Hu i in. 2020).

Powszechne użycie syntetycznych środków ochrony roślin (w tym insektycydów) prowadzi nie tylko do zanieczyszczenia środowiska oraz toksycznego wpływu na organizmy żywe. Dodatkowym bardzo poważnym problemem jest wzrastające zjawisko oporności stawonogów na stosowane insektycydy (Dannon i in. 2020). Jak podają Mantzoukas i Eliopoulos (2020) częste stosowanie syntetycznych środków ochrony roślin doprowadziło do uodpornienia się ponad 500 gatunków stawonogów na jedną lub więcej klas insektycydów.

Ze względu na ryzyko związane z coraz większym zanieczyszczeniem środowiska insektycydami syntetycznymi i ich wpływem na organizmy żywe, odchodzi się od ich stosowania a w zamian poszukuje się alternatywnych metod, które będą bardziej ekologiczne i przyjazne dla środowiska. Bezpieczną alternatywą dla chemicznych insektycydów są grzyby entomopatogenne. Charakteryzują się one zdolnością do uśmiercania stawonogów, co czyni je naturalnym czynnikiem kontroli populacji tych organizmów (Mascarin i Jaronski 2016; Lee i in. 2018; Litwin i in. 2020). Grzyby entomopatogenne są zaliczane do sześciu klas: *Ascomycota, Basidiomycota, Entomophtoromycota, Oomycetes, Chytridiomycota* oraz *Microsporidia*. Dotychczas poznano ponad 1600 gatunków tych mikroorganizmów, przy czym najczęściej występują gatunki należące do *Ascomycota* i *Entomophthoromycota* (Litwin i in. 2020).

Grzyby entomopatogenne infekują stawonogi poprzez bezpośrednią penetrację kutikuli i w odróżnieniu od bakterii i wirusów nie jest konieczne ich spożycie przez gospodarza (Bilgo i in. 2018). Proces infekcyjny rozpoczyna się od adhezji zarodników do kutikuli, co jest

możliwe dzięki działaniu sił hydrofobowych i elektrostatycznych oraz aktywności enzymów litycznych i białek zwanych hydrofobinami (Skinner i in. 2014; Litwin i in. 2020). Następnie dochodzi do kiełkowania zarodników, wytwarzania appressoriów i produkcji enzymów litycznych, które są kluczowym czynnikiem wirulencji grzybów entomopatogennych (Santi i in. 2010; Mondal i in. 2016; Litwin i in. 2020). Podczas procesu zakaźnego pierwszą barierą, którą musi pokonać grzyb entomopatogenny, jest zewnętrzna warstwa lipidowa oskórka owada, dlatego, podczas infekcji, lipazy wydzielane są jako pierwsze. Pod zewnętrzną warstwą lipidową oskórka znajdują się fibryle chityny osadzone w matrycy białkowej (Liu i in. 2007). Proteazy, które biorą udział w kolejnym etapie degradacji oskórka owadów, uważane są za najważniejsze enzymy w procesie zakaźnym (Mondal i in. 2016). Następnie, wytwarzane są liczne chitynazy, w tym endochitynazy (hydrolizujące wiązania β-1,4-glikozydowe) i egzochitynazy (hydrolizujące oligomery N-acetyloglukozaminy wytwarzane przez endochitynazy). Enzymy te rozkładają wspomniane wcześniej chitynowe fibryle, co razem z działaniem lipaz i proteaz umożliwia przeniknięcie grzyba do hemocelu stawonoga (Litwin i in. 2020). W kolejnym etapie procesu infekcyjnego zaczynają rosnąć strzępki grzyba, które kolonizują tkanki wewnętrzne stawonoga. Wytwarzane są także metabolity wtórne, powodujące paraliż i zakłócające procesy fizjologiczne żywiciela (Donzelli i Krasnoff 2016). Na wyniszczenie organizmu stawonoga podczas procesu infekcyjnego mają wpływ zarówno uszkodzenia mechaniczne jak i wykorzystywanie przez grzyba składników odżywczych znajdujących się w ciele ofiary (Gibson i in. 2014; Donzelli i Krasnoff 2016; Mascarin i Jaronski 2016; Fan i in. 2017b).

Grzyby entomopatogenne stosowane są jako bioinsektycydy w rolnictwie, głównie ekologicznym. Do produkcji biopreparatów bardzo często wykorzystywane są gatunki z rodzajów *Metarhizium* i *Beauveria*, które charakteryzują się szerokim spektrum działania (Castro i in. 2016; Ríos-Moreno i in. 2016; Gao i in. 2020). Oprócz zdolności infekcyjnych wobec stawonogów, grzyby te wykazują także inne istotne właściwości, pozwalające na wykorzystanie ich w procesach biotechnologicznych takich jak biosynteza nanocząstek (Różalska i in. 2016) czy biokonwersja steroidów (Xiong i in. 2006; Gonzalez i in. 2017). Udowodniono także, że organizmy te mogą być endofitami roślin wspomagającymi ich wzrost (Behie i Bidochka 2014; Ríos-Moreno i in. 2016).

Ponieważ głównym miejscem bytowania grzybów entomopatogennych jest gleba, są one narażone na kontakt z obecnymi w niej substancjami toksycznymi, takimi jak pestycydy. Drobnoustroje te wykształciły szereg mechanizmów przystosowawczych umożliwiających im przetrwanie w niesprzyjających warunkach (Szewczyk i in. 2018; Nowak i in. 2019).

Jak dotąd jedynie w nielicznych badaniach oceniano wpływ pyretroidów na grzyby entomopatogenne na poziomie komórkowym. Analizy te w większości koncentrowały się na zaburzeniach wzrostu grzybów w obecności tych substancji (Forlani i in. 2014; Abidin i in. 2017; Joshi i in. 2018; Chen i in. 2021). Brak natomiast jest doniesień skupiających się na mechanizmach oporności tych mikroorganizmów na insektycydy z grupy pyretroidów.

Beauveria bassiana, która stanowi przedmiot niniejszej rozprawy, odgrywa znaczącą rolę wśród grzybów entomopatogennych (Mascarin i Jaronski 2016) i jest uznawana za jeden z najskuteczniejszych bioinsektycydów (Dannon i in. 2020). Należy do entomopatogenów niespecyficznych – zwalcza zarówno liczne owady jak i roztocza (Ebani i Mancianti 2021), jest bardzo powszechna, gdyż występuje naturalnie we wszystkich typach gleb (Dannon i in. 2020) a ze względu na swoje właściwości wykorzystywana jest w biokontroli owadów na dużą skalę (Ebani i Mancianti 2021). Dodatkową zaletą *B. bassiana* jako czynnika biokontroli jest bezpieczeństwo stosowania tego gatunku, gdyż nie stanowi on zagrożenia dla zdrowia ludzi (Dannon i in. 2020).

Podjęcie badań zmierzających do poznania mechanizmów oporności grzybów entomopatogennych na insektycydy chemiczne wydaje się być istotnym problemem ze względu na różnorodne funkcje ekologiczne pełnione przez te mikroorganizmy i jest niezwykle istotne biorąc pod uwagę rosnące zanieczyszczenie środowiska naturalnego.

II. Cele pracy

Przewodnim celem niniejszej rozprawy doktorskiej jest określenie mechanizmów oporności grzyba entomopatogennego *Beauveria bassiana* ARSEF 2860, pochodzącego z Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (USA) na insektycydy z grupy pyretroidów - λ-cyhalotrynę, α-cypermetryne oraz deltametrynę.

Ogólny cel badawczy realizowano za pomocą celów szczegółowych, za które przyjęto:

1. Ocena wpływu pyretroidów na wzrost, aktywność metaboliczną oraz zarodnikowanie *B. bassiana.*
2. Określenie zdolności *B. bassiana* do akumulacji pyretroidów w grzybni.
3. Ocena zmian adaptacyjnych *B. bassiana* wywołanych przez pyretroidy.
4. Określenie zmian zachodzących w profilu fosfolipidowym oraz w zawartości lipidów neutralnych w komórkach *B. bassiana* wywołanych przez pyretroidy.
5. Ocena wpływu pyretroidów na proteom zewnątrzkomórkowy *B. bassiana* oraz aktywność enzymów biorących udział w procesie infekcyjnym grzybów entomopatogennych.
6. Określenie wpływu pyretroidów na proteom wewnątrzkomórkowy *B. bassiana.*
7. Ocena poziomu stresu oksydacyjnego w komórkach *B. bassiana* wywoływanego przez pyretroidy.

III. Materiały i techniki wykorzystane podczas realizacji rozprawy doktorskiej

Modelem badawczym podczas realizacji pracy doktorskiej był szczep grzyba entomopatogennego *Beauveria bassiana* ARSEF 2860 uzyskany z Agricultural Research Service Collection of Entomopathogenic Fungal Cultures, USA.

W badaniach wykorzystano syntetyczne insektycydy z grupy pyretroidów: λ-cyhalotrynę, α-cypermetrynę oraz deltametrynę. W celu uzyskania końcowych stężeń pyretroidów wynoszących odpowiednio 5, 50 i 100 mg L^{-1}, badane substancje dodawano do hodowli z roztworu wyjściowego 10 mg mL^{-1} w acetonitrylu.

Poniżej przedstawiono metody badawcze zastosowane podczas realizacji niniejszej pracy doktorskiej:

- Spektrofluorymetrię zastosowano
  - w analizach wpływu insektycydów na aktywność metaboliczną (P2),
  - w badaniach wpływu insektycydów na przepuszczalność błon komórkowych (P2).
- Spektrometrię wykorzystano w celu oznaczenia aktywności
  - enzymów litycznych (P3),
  - enzymów antyoksydacyjnych (katalaza, dysmutaza ponadtlenkowa) (P3),
  - lakazy oraz wewnątrzkomórkowej fosfolipazy C (P3)
  - a także w celu pomiaru stężenia wyizolowanych białek (P3).
- Mikroskopię konfokalną oraz świetlną wykorzystano
  - do oznaczenia zawartości wolnych rodników wytwarzanych w obecności pyretroidów (P2 i P3)
  - a także w celu określenia wpływu insektycydów na morfologię strzępek oraz zdolność *B. bassiana* do wytwarzania blastospor (P2).
- Chromatografię gazową (GC-MS) wykorzystano do oznaczenia
  - ilości insektycydów pozostałych w podłożu pohodowlanym oraz zakumulowanych w grzybni *B. bassiana* (P2 i P3)
  - a także ilości produkowanego przez *B. bassiana* ergosterolu (P2).
- Absorpcyjną spektrometrię atomową (AAS) wykorzystano do oznaczenia zawartości metali w grzybni *B. bassiana* (P2).
- Chromatografię cieczową (LC-MS/MS) zastosowano do określenia wpływu pyretroidów
  - na profil fosfolipidowy (P3),

    - na zawartość lipidów neutralnych (P2 i P3)
    - oraz do ilościowej analizy metabolitu wtórnego (oosporeiny) produkowanego przez *B. bassiana* (P2).
- Elektroforezę 1-D oraz 2-D wykorzystano w celu przeprowadzenia rozdziału białek zewnątrz- i wewnątrzkomórkowych *B. bassiana* (P3).
- Spektrometrię mas MALDI-TOF/TOF wykorzystano w celu identyfikacji białek *B. bassiana* wyizolowanych z biomasy oraz podłoża pohodowlanego (P3).

IV. Realizacja celów pracy

IV.1. Ocena wpływu pyretroidów na wzrost, aktywność metaboliczną oraz zarodnikowanie *B. bassiana*

W pierwszym etapie badań oceniono wpływ trzech insektycydów z grupy pyretroidów (λ-cyhalotryny, α-cypermetryny oraz deltametryny) na wzrost, morfologię strzępek, zdolność do wytwarzania blastospor oraz aktywność metaboliczną grzyba entomopatogennego *B. bassiana.* Wykazano, że badane substancje w stężeniach 50 i 100 mg L^{-1} powodowały spadek zawartości suchej masy po 36 godzinach inkubacji. W 48-godzinnych hodowlach istotny statystycznie spadek suchej masy odnotowano jedynie w próbach z dodatkiem λ-cyhalotryny i α-cypermetryny w stężeniu 100 mg L^{-1}. Podczas obserwacji mikroskopowych grzybni nie wykazano zmian morfologicznych spowodowanych obecnością pyretroidów. Stwierdzono natomiast istotny spadek liczby blastospor w hodowlach 24- i 48-godzinnych zawierających badane substancje w stężeniach 50 i 100 mg L^{-1}. Zaobserwowany niekorzystny wpływ pyretroidów na tworzenie blastospor w hodowlach płynnych został opisany po raz pierwszy w pracy P2 (Litwin i in. 2021). W literaturze dostępne są dane potwierdzające, że cypermetryna i deltametryna (Natali de Oliveira i in. 2003) a także niektóre herbicydy (Kos i Celar 2013) hamują sporulację u *B. bassiana* na pożywkach stałych.

W tej części badań stwierdzono również, że najwyższe badane stężenie pyretroidów (100 mg L^{-1}) nie wpływa negatywnie na aktywność metaboliczną *B. bassiana*, a nawet powoduje jej wzrost w hodowlach 24-godzinnych. Jest to interesujący wynik, ponieważ badane substancje (w stężeniach 50 i 100 mg L^{-1}) wpływały niekorzystnie na ilość wytwarzanej biomasy badanego drobnoustroju w 36- i 48-godzinnych hodowlach. Większość dotychczas badanych związków toksycznych powoduje jednoczesne zahamowanie wzrostu oraz żywotności mikroorganizmów. W badaniach opublikowanych przez Różalską i in. (2014) stwierdzono, że obecność 4-*n*-nonylofenolu oraz technicznego nonylofenolu powodowała spadek aktywności metabolicznej innego grzyba entomopatogennego – *Metarhizium robertsii*. Grzyby mikroskopowe posiadają mechanizmy warunkujące ich przetrwanie w obecności toksycznych zanieczyszczeń (Litwin i in. 2020). Wyniki uzyskane w tej części pracy stanowiły punkt wyjścia dla kolejnych badań mających na celu poznanie mechanizmów oporności występujących u grzybów entomopatogennych w obecności insektycydów z grupy pyretroidów.

IV.2. Określenie zdolności *B. bassiana* do akumulacji pyretroidów w grzybni

W pracach P2 (Litwin i in. 2021) oraz P3 (Litwin i in. 2023) wchodzących w skład rozprawy doktorskiej, po raz pierwszy wykazano, że pyretroidy (λ-cyhalotryna, α-cypermetryna oraz deltametryna) akumulują się w grzybni *B. bassiana.* Stwierdzono, że badany grzyb entomopatogenny wykazuje zdolność do akumulacji tych substancji już w drugiej dobie inkubacji i efekt ten utrzymuje się w 7 i 14 dobie (dla wszystkich trzech badanych pyretroidów) oraz w 21 dobie inkubacji (dla α-cypermetryny i deltametryny).

Przedstawione przez nas wyniki mają nowatorski charakter, ponieważ dostępne dotąd dane wskazują na zdolność do akumulacji pyretroidów jedynie w tkankach roślinnych i zwierzęcych (Tang i in. 2018). We wcześniejszych badaniach wykazano także zdolność *Metarhizium sp*. do akumulacji insektycydów neonikotynoidowych (Nowak i in. 2020) co może sugerować, że zjawisko akumulacji insektycydów przez grzyby entomopatogenne jest dość powszechne, choć do tej pory rzadko opisywane. W związku z zaobserwowaną akumulacją pyretroidów w grzybni *B. bassiana* przeprowadzono szczegółowe badania, mające na celu określenie mechanizmów oporności tego grzyba entomopatogennego na insektycydy z grupy pyretroidów.

IV.3. Ocena zmian adaptacyjnych u *B. bassiana* wywoływanych przez pyretroidy

W celu poznania mechanizmów oporności *B. bassiana* na pyretroidy, poszukiwano zmian adaptacyjnych zachodzących u tego grzyba w obecności badanych insektycydów. Oznaczono zawartość jonów metali, ergosterolu, acyloglicerolu, poziom oosporeiny, wewnątrzkomórkowej fosfolipazy C oraz wpływ tych insektycydów na przepuszczalność błon komórkowych *B. bassiana*.

Zbadano wpływ zakumulowanych pyretroidów, dodawanych w stężeniach początkowych 5, 50 i 100 mg L^{-1} na zawartość metali w biomasie *B. bassiana.* Po raz pierwszy dla grzybów wykazano, że wszystkie badane pyretroidy powodowały spadek zawartości sodu w komórkach tego grzyba entomopatogennego (Litwin i in. 2021). Wykazano także spadek zawartości innych pierwiastków w komórkach *B. bassiana*: potasu, magnezu i wapnia w obecności α-cypermetryny w stężeniu 5 mg L^{-1} oraz deltametryny w stężeniu 50 mg L^{-1}.

Nadmierny wypływ jonów może prowadzić do zaburzenia podstawowych funkcji, takich jak synteza białek czy aktywacja enzymów, dla których konieczne jest wysokie stężenie jonów potasu (Benito i in. 2011). Jak do tej pory nie ma doniesień literaturowych wskazujących na możliwe zaburzenia transportu jonowego u grzybów pod wpływem pyretroidów.

Wyniki naszych badań dowiodły także, że pyretroidy wpływają na zawartość ergosterolu w biomasie *B. bassiana*. Ergosterol jest ważnym składnikiem komórek grzybowych i stanowi istotny marker odpowiedzi komórek na substancje toksyczne oraz wpływa na integralność i funkcjonowanie błon komórkowych (Suchodolski i in. 2019). Obecność λ-cyhalotryny (we wszystkich badanych stężeniach) oraz deltametryny w stężeniu 50 mg L^{-1} powodowała spadek zawartości ergosterolu, natomiast obecność α-cypermetryny (we wszystkich badanych stężeniach) oraz deltametryny w stężeniu 5 mg L^{-1} powodowała wzrost zawartości tego steroidu. W piśmiennictwie brakuje doniesień na temat wpływu pyretroidów na zawartość ergosterolu w komórkach grzybów. Wyniki przeprowadzonych badań dotyczących zawartości jonów metali i ergosterolu wskazują na fakt, że pyretroidy wpływają na błony komórkowe *B. bassiana*.

Badania przepuszczalności osłon komórkowych z wykorzystaniem jodku propidu wskazują, że obecność λ-cyhalotryny i α-cypermetryny w stężeniu 100 mg L^{-1} powoduje wzrost przepuszczalności błon komórkowych *B. bassiana*, co może być powiązane z opisywanymi zaburzeniami równowagi jonowej (Litwin i in. 2021). Wzrost przepuszczalności błon u grzybów mikroskopowych w obecności substancji toksycznych zaobserwowali także Nykiel-Szymańska i in. (2019), którzy badali *Trichoderma* spp. w obecności herbicydów chloroacetanilidowych oraz Bernat i in. (2018), którzy potwierdzili wzrost przepuszczalności błon u grzyba *Umbelopsis isabellina* w obecności 2,4-D. Zwiększona płynność błon ułatwia transport substancji toksycznych do komórek grzybów, co umożliwia ich akumulację oraz degradację (Stolarek i in. 2019). W pracach P2 i P3 wykazano, że pyretroidy są akumulowane we wnętrzu komórek *B. bassiana* co może być powiązane z zaburzeniami przepuszczalności osłon komórkowych.

W odpowiedzi na niekorzystne warunki środowiskowe, grzyby entomopatogenne wydzielają metabolity wtórne, czyli związki organiczne, które charakteryzują się niską masą cząsteczkową (Litwin i in. 2020). Metabolity wtórne wytwarzane przez grzyby wspomagają utrzymanie funkcji życiowych oraz biorą udział w ochronie przed stresem biotycznym i abiotycznym (Mc Namara i in. 2019; Litwin i in. 2020), ponadto w przypadku grzybów entomopatogennych substancje te wspomagają proces infekcyjny (Donzelli i Krasnoff 2016; Litwin i in. 2020). Oosporeina jest metabolitem wtórnym należącym do grupy dibenzochinonów, który wykazuje działanie owadobójcze, przeciwwirusowe i przeciwbakteryjne (Feng i in. 2015). Przeprowadzone analizy HPLC-MS/MS wskazują, że obecność λ-cyhalotryny, α-cypermetryny oraz deltametryny w stężeniu 100 mg L^{-1} znacznie hamuje produkcję

oosporeiny u grzyba entomopatogennego *B. bassiana* w 36-godzinnych hodowlach, ale nie wpływa na zawartość tego metabolitu w hodowlach 48-godzinnych. Obserwacje te stanowią kolejne potwierdzenie negatywnego wpływu pyretroidów na grzyby entomopatogenne. Czasowe zahamowanie produkcji oosporeiny może wynikać zarówno z nieznacznego ograniczenia wzrostu grzyba jak i pojawiającego się stresu oksydacyjnego. W efekcie, zaobserwowana zmiana w produkcji tego metabolitu może prowadzić do zmniejszenia zdolności infekcyjnych *B. bassiana* (Litwin i in. 2020). W pracy P2 po raz pierwszy opisano negatywny wpływ insektycydów na produkcję oosporeiny. W piśmiennictwie dostępne są nieliczne dane opisujące wpływ insektycydów na wytwarzanie innych metabolitów wtórnych. W badaniach Nowak i in. (2020) wykazano spadek produkcji destruksyn przez różne gatunki grzyba entomopatogennego *Metarhizium* w obecności insektycydu neonikotynoidowego acetamiprydu.

W pracy P2 oznaczono także wpływ pyretroidów na produkcję wewnątrzkomórkowej fosfolipazy C. Enzym ten jest zaangażowany w metabolizm grzybów entomopatogennych, m.in. katalizuje hydrolizę składnika błony komórkowej - 4,5-bisfosforanu fosfatydyloinozytolu (PIP2) wytwarzając dwa przekaźniki - 4,5-trifosforan inozytolu (IP3) oraz diacyloglicerol (DAG). IP3 wspomaga uwalnianie jonów wapnia Ca^{2+} z wewnątrzkomórkowych zapasów, natomiast DAG indukuje aktywność kinazy białkowej C (PKC), biorącej udział w transdukcji sygnału (Barman i in. 2018). Ponadto, wewnątrzkomórkowa fosfolipaza C podnosi odporność grzybów na stres oksydacyjny (Tisi i in. 2002; Djordjevic 2010; Barman i in. 2018) oraz wpływa na wzrost, rozwój strzępek i sporulację a także wspomaga ich procesy infekcyjne, poprzez pozytywny wpływ na rozprzestrzenianie się grzyba w hemocelu owada oraz pokonywanie mechanizmów odpornościowych gospodarza (Djordjevic 2010). Wyniki uzyskane w tej części badań wskazują, że wpływ pyretroidów na aktywność wewnątrzkomórkowej fosfolipazy C zależy zarówno od użytej substancji jak i od czasu inkubacji. Wykazano, że obecność wszystkich badanych pyretroidów w hodowlach 48-godzinnych oraz obecność α-cypermetryny i deltametryny w hodowlach 168-godzinnych powodowały spadek aktywności fosfolipazy, natomiast obecność λ-cyhalotryny w hodowlach 120-godzinnych powodowała wzrost aktywności tego enzymu.

Wpływ pyretroidów na aktywność fosfolipazy C znajduje odzwierciedlenie w zaburzeniach wspomnianych wcześniej funkcji i procesów, w których uczestniczy ten enzym. Uzyskane wyniki stanowią zatem kolejne potwierdzenie, że zakumulowane pyretroidy zaburzają metabolizm i zdolności infekcyjne *B. bassiana*.

IV.4. Określenie zmian zachodzących w profilu fosfolipidowym oraz w zawartości lipidów neutralnych w komórkach *B. bassiana* podczas akumulacji pyretroidów

Określenie zmian zachodzących w profilu fosfolipidowym jest wiarygodnym wskaźnikiem stanu, w jakim znajduje się organizm. Fosfolipidy odpowiadają za kondycję błon komórkowych i odgrywają pośrednią rolę w metabolizmie podstawowym (Bernat i in. 2014). Stwierdzono, że najważniejszymi klasami fosfolipidów u *B. bassiana* są fosfatydylocholiny (PC, w ilości 51,08 %) i fosfatydyloetanoloaminy (PE, 45,48 %). Ilość fosfatydyloinozytolu (PI), fosfatydyloseryny (PS) i kwasu fosfatydowego (PA) wynosi odpowiednio 2,78%, 0,58% i 0,08%. Przedstawione w pracy P2 wyniki są zbieżne z danymi literaturowymi np. Palma-Guerrero i in. (2010), w których także wykazano, że PC i PE są dominującymi klasami fosfolipidów w błonach *B. bassiana.*

Dane lipidomiczne opublikowane w pracy P2 wskazują, że wszystkie badane pyretroidy w stężeniu 100 mg L^{-1} wpływają na profil fosfolipidowy *B. bassiana*. Stwierdzono, że badane substancje powodują wzrost PC przy jednoczesnym spadku PE. Ponieważ PC i PE są głównymi fosfolipidami w komórkach *B. bassiana,* dynamika błony komórkowej zależy od ich proporcji, a wzrastający stosunek PC/PE prowadzi do zwiększenia płynności błony komórkowej (Stolarek i in. 2019). Zaobserwowano również, że w obecności pyretroidów wzrasta zawartość PI, który pełni rolę prekursora wielu związków lipidowych, dzięki czemu odgrywa kluczową rolę w metabolizmie podstawowym. Nie zaobserwowano istotnych zmian w zawartości PS ani PA.

Kolejnym bardzo ważnym punktem niniejszej rozprawy było określenie zmian ilościowych lipidów neutralnych w obecności pyretroidów. W tym celu zastosowano dwa różne stężenia wyjściowe pyretroidów – 100 mg L^{-1} w hodowlach 48-godzinnych (Litwin i in. 2021) oraz 5 mg L^{-1} w hodowlach 24-, 48-, 120- oraz 168-godzinnych (Litwin i in. 2023). Stwierdzono, że wpływ pyretroidów na zawartość neutralnych lipidów zależy od zastosowanego stężenia tych substancji.

Zaobserwowano, że akumulacja pyretroidów, dodawanych w stężeniu 100 mg L^{-1} spowodowała spadek ilości neutralnych lipidów w 48-godzinnych hodowlach *B. bassiana*. Po raz pierwszy wykazano, że zawartość diacylogliceroli (DAG) przewyższała zawartość triacylogliceroli (TAG) oraz że dodatek α-cypermetryny powodował największy spadek zawartości TAG, a dodatek λ-cyhalotryny – DAG. W literaturze naukowej brakuje doniesień dotyczących szkodliwego wpływu pyretroidów na syntezę acylogliceroli u grzybów entomopatogennych. Dostępne dane literaturowe dotyczą innych organizmów np. małży i wskazują na odwrotny efekt - wzrost zawartości acylogliceroli u małży *Donax trunculus* w obecności pyretroidów (Aouini i in. 2017).

W pracy P3 wykazano, że u *B. bassiana* całkowita zawartość acyloglicerolі zmniejszała się wraz z czasem hodowli – pomiędzy 24 a 168 godziną hodowli stwierdzono 3-krotny spadek zawartości TAG oraz ponad 11-krotny spadek zawartości DAG w kontrolach biotycznych. Wykazano również, że akumulacja α-cypermetryny i deltametryny dodawanych w stężeniu 5 mg L^{-1} powodowała wzrost całkowitej zawartości TAG. Efekt ten dla α-cypermetryny zauważono w hodowlach 24- i 120-godzinnych, natomiast dla deltametryny – w hodowlach 48-godzinnych. Jest to efekt odwrotny od tego, który zaobserwowano dla pyretroidów w stężeniu 100 mg L^{-1}. Dodatek λ-cyhalotryny w stężeniu 5 mg L^{-1} nie wpływał na całkowitą zawartość TAG, ale powodował spadek całkowitej zawartości DAG. Z kolei obecność α-cypermetryny i deltametryny nie wpływała na całkowitą zawartość DAG.

Obserwowany wzrost całkowitej zawartości TAG przy stabilnej zawartości DAG w hodowlach z dodatkiem α-cypermetryny i deltametryny w stężeniu 5 mg L^{-1} może być spowodowany spadkiem aktywności wewnątrzkomórkowej fosfolipazy C, która odpowiada za hydrolizę wiązań fosfodiestrowych w szkielecie fosfolipidowym, co prowadzi do przekształcania TAG do DAG (Santi i in. 2010).

W niniejszej rozprawie, w grzybni *B. bassiana,* zidentyfikowano 13 gatunków TAG i 9 gatunków DAG oraz stwierdzono, że dodatek pyretroidów powoduje zmiany w zawartości poszczególnych lipidów obojętnych. Wykazano, że dodatek λ-cyhalotryny i α-cypermetryny w stężeniu 100 mg L^{-1} powodował wzrost zawartości nasyconych tri- i diacyloglicerolі oraz spadek nienasyconych triacyloglicerolі. Najniższy stosunek TAG/DAG odnotowano w próbach z dodatkiem α-cypermetryny w stężeniu 100 mg L^{-1}. W przypadku hodowli z dodatkiem pyretroidów w stężeniu 5 mg L^{-1} stwierdzono, że największy wpływ na poszczególne acyloglicerole wykazywała zakumulowana α-cypermetryna.

Acyloglicerole pełnią wiele istotnych funkcji u grzybów entomopatogennych: stanowią główny materiał rezerwowy komórek, są ważnym elementem szlaku biosyntezy fosfolipidów, biorą udział w ochronie komórek przed stresem oksydacyjnym zapobiegając wytwarzania się ROS oraz wywierają wpływ na zjadliwość tych organizmów – ułatwiają penetrację kutikuli owada (Gao i in. 2016). Prawidłowy metabolizm acyloglicerolі jest niezbędny dla wewnątrzkomórkowej homeostazy i równowagi energetycznej organizmów żywych (Fan i in. 2017a). Ponieważ acyloglicerole biorą udział w procesach niezbędnych do przetrwania grzybów w środowisku naturalnym, wykazanie, że akumulacja pyretroidów powoduje zmiany w ilości lipidów neutralnych wskazuje, że substancje te wywierają istotny wpływ na *B. bassiana*.

IV.5. Ocena wpływu pyretroidów na proteom zewnątrzkomórkowy *B. bassiana* oraz aktywność enzymów biorących udział w procesie infekcyjnym grzybów entomopatogennych

Bardzo istotną część pracy stanowiła ocena wpływu pyretroidów na proteom badanego grzyba entomopatogennego. Białka wyizolowano z biomasy oraz podłoża pohodowlanego pochodzących z 48- oraz 168-godzinnych hodowli *B. bassiana* z dodatkiem λ-cyhalotryny, α-cypermetryny oraz deltametryny w stężeniu 5 mg L^{-1}. Białka zewnątrzkomórkowe rozdzielano metodą elektroforezy jednokierunkowej a uzyskane wyniki przedstawiono w sposób jakościowy.

W zewnątrzkomórkowym proteomie *B. bassiana* wykazano obecność enzymów zaangażowanych w proces infekcyjny: zewnątrzkomórkowej fosfolipazy C, fosfoesterazy, licznych proteaz (w tym tripeptydylopeptydazy Sed1, alkalicznej a także trypsynopodobnej proteazy serynowej oraz metaloproteazy), chitynazy oraz endo-beta-N-acetylglukozaminidazy, a także lakazy, która również może brać udział w procesie infekcyjnym.

Enzymy lipolityczne biorą udział w degradacji zewnętrznych warstw naskórka owada i odpowiadają za hydrolizę wiązań estrowych lipidów i wosków (Pedrini i in. 2007; Dhawan i Joshi 2017; Litwin i in. 2020). Z kolei, zewnątrzkomórkowa fosfolipaza C odpowiada za hydrolizę wiązań fosfodiestrowych i degradację fosfolipidów błon komórkowych owada podczas procesu zakaźnego (Santi i in. 2010). W kolejnym etapie degradacji naskórka owadów uczestniczą proteazy, które uważane są za najważniejsze enzymy w procesie zakaźnym (Mondal i in. 2016; Wang i in. 2021). Mogą one również pełnić dodatkową rolę – zapewniać inaktywację owadzich białek o właściwościach przeciwgrzybiczych (Sotelo-Mundo i in. 2007). Enzymy chitynolityczne grzybów entomopatogennych oprócz degradacji naskórka owadów, przeprowadzają również przebudowę ścian komórkowych strzępek podczas ich wzrostu i rozgałęziania (Mondal i in. 2016; Lovera i in. 2020). Lakazy grzybicze zapewniają eliminację ROS wydzielanych przez owady podczas procesu infekcyjnego (Lu i in. 2021).

Na podstawie wyników analiz proteomicznych, postanowiono także określić wpływ pyretroidów na aktywność enzymów litycznych *B. bassiana* po 48, 120 i 168 godzinach inkubacji. Wykazano, że α-cypermetryna i λ-cyhalotryna podnosiły aktywność lipolityczną oraz ogólną aktywność proteolityczną, jednocześnie obniżając aktywności endoproteazy serynowej (PR1) oraz kwaśnej proteazy trypsynopodobnej (PR2), które są uznawane za kluczowe czynniki wirulencji. Zakumulowana deltametryna, w 120 godzinie hodowli, powodowała spadek aktywności proteazy PR2 oraz wzrost aktywności proteaz PR1 i PR2

po 168 godzinach hodowli. Wyniki uzyskane w tej części badań, wskazują, że zakumulowane pyretroidy powodują wzrost ogólnej aktywności proteolitycznej przy jednoczesnym spadku aktywności proteaz PR1 i PR2. Jest to zgodne z analizami proteomicznymi, które wykazały, że w proteomie *B. bassiana* obecne są proteazy należące do różnych rodzin, które odpowiadają za ogólną aktywność proteolityczną. W pracy Santi i in. (2010) zasugerowano, że zróżnicowany zestaw proteaz u grzyba *M. anisopliae* zwiększa jego zdolności adaptacyjne do różnych warunków środowiskowych.

W dysertacji, zbadano także wpływ pyretroidów na aktywność enzymów chitynolitycznych *B. bassiana* - endochitynazy (triacetylochitotriozydazy) i dwóch egzochiotynaz (chitobiozydazy i N-acetyloglukozaminidazy). Otrzymane wyniki (P3) wskazują, że aktywność tych enzymów różni się w zależności od użytej substancji oraz od czasu inkubacji. W 48-godzinnych hodowlach λ-cyhalotryna powodowała wzrost aktywności wszystkich badanych enzymów chitynolitycznych, deltametryna powodowała wzrost aktywności chitobiozydazy a α-cypermetryna – wzrost aktywności N-acetyloglukozaminidazy. W 168-godzinnych hodowlach zaobserwowano spadek aktywności triacetylochitotriozydazy w obecności wszystkich trzech pyretroidów, natomiast insektycydy nie wpływały na aktywność egzochitynaz. Wykazano również, że obecność pyretroidów powoduje wzrost aktywności lakazy – efekt ten zaobserwowano w 48-godzinnych hodowlach z dodatkiem λ-cyhalotryny i α-cypermetryny oraz w 120-godzinnych hodowlach z dodatkiem α-cypermetryny i deltametryny. Na podstawie wyników uzyskanych w tej części badań stwierdzono, że λ-cyhalotryna i α-cypermetryna powodują wzrost aktywności enzymów zaangażowanych w proces infekcyjny.

Podczas analiz proteomicznych, wykazano także obecność innych białek zewnątrzkomórkowych, których synteza była indukowana dodatkiem pyretroidów. W hodowlach z deltametryną zaobserwowano obecność antygenowego białka podobnego do taumatyny, które wykazuje aktywność przeciwgrzybiczą i pozwala na eliminację konkurencji między różnymi gatunkami grzybów entomopatogennych podczas procesu infekcyjnego oraz chroni je przed innymi grzybami chorobotwórczymi (Liu i in. 2010). Obecność λ-cyhalotryny i α-cypermetryny indukowała wytwarzanie białek odpowiedzialnych za wzrost strzępek i syntezę ściany komórkowej: glukanozylotransferazy, glukan endo-1,6-beta-glukozydazy B, beta-1,6-glukanazy oraz 1,3-beta-glukozydazy glukanowej. Uzyskane w rozprawie wyniki sugerują, że białka te są niezbędne do naprawy ścian komórkowych uszkadzanych przez zakumulowane pyretroidy. Również niektóre ze wspomnianych wcześniej

proteaz (metaloproteaza StcE, peptydaza S1 i S6, chymotrypsyna/Hap) zostały zidentyfikowane jedynie w próbach zawierających α-cypermetrynę i deltametrynę.

IV.6. Ocena wpływu pyretroidów na proteom wewnątrzkomórkowy *B. bassiana*

Wyniki analiz proteomicznych umożliwiających określenie zmian zachodzących w wewnątrzkomórkowym proteomie *B. bassiana* w obecności zakumulowanych pyretroidów stanowią istotną część rozprawy doktorskiej i zostały opisane w pracy P3 (Litwin i in. 2023). W celu identyfikacji białek wewnątrzkomórkowych wykorzystano techniki proteomiczne z użyciem spektrometru masowego MALDI TOF/TOF.

IV.6.1. Białka *B. bassiana* powiązane z akumulacją pyretroidów

Wykazano, że dodatek α-cypermetryny indukował wytwarzanie białek zaangażowanych w metabolizm związków azotowych. Były to: hydrataza cyjanianowa, hydrataza cyjankowa oraz flawohemoproteina. Enzymy te pełnią w komórkach funkcję ochronną - hydrataza cyjankowa przekształca obecną w cypermetrynie grupę cyjankową (Nolan i in. 2003; Martínková i in. 2015) a flawohemoproteina bierze udział w ochronie organizmów przed toksycznym wpływem związków azotowych podczas stresu nitrozacyjnego (Hausladen i in. 1998).

Obecność α-cypermetryny w próbach skutkuje także nadprodukcją białek biorących udział w organizacji cytoszkieletu aktynowego: kofiliny, białka wiążącego aktynę (actin lateral binding protein) oraz podjednostki białka blokującego F-aktynę. Kofilina reguluje dynamikę filamentów aktynowych i wiąże się z monomerami aktyny, białka wiążące aktynę natomiast przyłączają się do filamentów aktynowych powodując redukcję ich wydłużania, co przyczynia się do zwiększenia puli monomerów aktyny (Kim i in. 2021). Obecność tych enzymów może tłumaczyć brak zmian morfologicznych w strzępkach *B. bassiana* (Litwin i in. 2021) przy jednoczesnej nadprodukcji enzymów szlaków metabolicznych w hodowlach 168-godzinnych z dodatkiem α-cypermetryny.

Wykazano również, że deltametryna w 48-godzinnych hodowlach indukowała produkcję dehydrogenazy alkoholowej i hamowała jej produkcję w hodowlach 168-godzinnych. Dehydrogenaza alkoholowa jest zaangażowana w metabolizm nie tylko alkoholi, ale również aldehydów, ketonów oraz innych ksenobiotyków (Łaniewska-Dunaj i in. 2013) a w hodowlach *B. bassiana* z dodatkiem deltametryny może odpowiadać za jej detoksykację.

IV.6.2. Wpływ pyretroidów na enzymy głównych szlaków metabolicznych *B. bassiana*

Przeprowadzone analizy proteomiczne *B. bassiana* wykazały, że pyretroidy wpływają także na enzymy zaangażowane w główne szlaki metaboliczne – glikolizę, Cykl Krebsa, Cykl Pentozofosforanowy oraz Łańcuch Transportu Elektronów (ETC).

W próbach z dodatkiem λ-cyhalotryny zaobserwowano zmniejszenie produkcji enzymów biorących udział w glikolizie, Cyklu Krebsa i Szlaku Pentozofosforanowym. Wpływ tej substancji na enzymy zaangażowane w ETC zależał od długości trwania hodowli – po 48 godzinach inkubacji dodatek λ-cyhalotryny hamował, natomiast po 168 godzinach – indukował produkcję tych enzymów. W przypadku α-cypermetryny, zaobserwowano nadprodukcję enzymów zaangażowanych w procesy: glikolizy, Cyklu Krebsa, Szlaku Pentozofosforanowego oraz ETC po 168 godzinach hodowli. Efekt zaobserwowany w początkowych godzinach inkubacji najprawdopodobniej jest mechanizmem obronnym organizmu powiązanym z obecnością tego pyretroidu. Wykazano również, że spowolniona akumulacja deltametryny spowodowała opóźnienie jej wpływu na metabolizm podstawowy. W początkowych godzinach hodowli z dodatkiem tej substancji obserwowano nadprodukcję enzymów biorących udział w glikolizie, Cyklu Krebsa, Cyklu Pentozofosforanowym i ETC, natomiast po 168 godzinach odnotowano obniżenie produkcji enzymów zaangażowanych w te procesy.

Wykazano również, że badane substancje zaburzają działanie mitochondriów, odpowiedzialnych za produkcję ATP. W 48-godzinnych hodowlach zaobserwowano całkowite zahamowanie produkcji enzymu kompleksu cytochromu b-c1 (III) (podjednostka 7). Akumulacja deltametryny wpływała także na wytwarzanie syntazy ATP – w początkowych godzinach hodowli nastąpiła nadprodukcja, natomiast w 168-godzinnych hodowlach obniżenie produkcji tego enzymu.

IV.6.3. Wpływ pyretroidów na białka stresu oksydacyjnego u *B. bassiana*

Analizy proteomiczne umożliwiły określenie wpływu pyretroidów na wytwarzanie enzymów odpowiedzi na stres oksydacyjny u *B. bassiana*. Wykazano, że peroksydaza glutationowa (GPx) występowała jedynie w 168-godzinnych próbach z dodatkiem pyretroidów, co wskazuje na fakt, że substancje te indukowały jej wytwarzanie. GPx odpowiada za redukcję nadtlenków (podczas której następuje przekształcenie zredukowanych cząsteczek glutationu w dwusiarczek glutationu) i należy do układu glutationowego, jednego z najważniejszych systemów antyoksydacyjnych w komórkach (Huarte-Bonnet i in. 2015). Kolejnym enzymem

zaangażowanym w ochronę komórek przed stresem oksydacyjnym jest S-transferaza glutationowa (GST), która usuwa glutation z substratów i prowadzi do jego sprzęgania ze związkami posiadającymi grupy elektrofilowe, co prowadzi do powstania nieszkodliwych pochodnych (Morel i in. 2009; Calmes i in. 2015; Huarte-Bonnet i in. 2015). Wykazano, że obecność pyretroidów powoduje obniżenie produkcji GST w 48-godzinnych hodowlach. W 168-godzinnych hodowlach natomiast odnotowano obecność tego enzymu jedynie w obecności α-cypermetryny. W literaturze naukowej dostępne są informacje o roli GST w odpowiedzi na stres oksydacyjny i adaptacji do niesprzyjających warunków środowiskowych – wykazano, że u grzyba *Sclerotinia sclerotiorum*, podczas procesu infekcyjnego, a także w obecności syntetycznych izotiocyjanianów, nastąpiła indukcja genów kodujących GST oraz zwiększona aktywność transferazy (Rahmanpour i in. 2009).

W przeprowadzonych w dysertacji badaniach zaobserwowano również nadprodukcję peroksyredoksyny oraz białek szoku cieplnego w 48-godzinnych hodowlach z dodatkiem deltametryny. Peroksyredoksyny uważane są za jeden z najważniejszych komórkowych systemów obronnych przed stresem oksydacyjnym (Boysen i in. 2021), redukują szerokie spektrum reaktywnych form tlenu (Rocha i in. 2018), biorą udział w transdukcji sygnałów (Breitenbach i in. 2015) a także nasilają zjadliwość grzybów (Boysen i in. 2021).

Białka szoku cieplnego (Heat Shock Protein – HSP) uznawane są za markery stresu oksydacyjnego, ich zawartość wzrasta w obecności substancji toksycznych a także w warunkach niedoboru tlenu i azotu (Soboń i in. 2019). HSP chronią komórki przed uszkodzeniami modyfikując ich aktywność metaboliczną oraz wspomagają potencjał infekcyjny *B. bassiana* (Xu i in. 2013; Wang i in. 2020).

Wykazano, że deltametryna w 168-godzinnych hodowlach wpływa hamująco na wytwarzanie białka domeny WSC wiążącego węglowodany. Białko to jest odpowiedzialne za utrzymanie integralności ściany komórkowej (Oide i in. 2019).

IV.6.4. Wpływ pyretroidów na produkcję białek opiekuńczych u *B. bassiana*

Przeprowadzone analizy proteomiczne wskazują, że badane insektycydy wpływają na produkcję białek opiekuńczych i innych białek biorących udział w metabolizmie białek. Zarówno w hodowlach 48- jak i 168-godzinnych zawierających pyretroidy zaobserwowano zmniejszenie produkcji cyklofiliny A, natomiast w hodowlach 168-godzinnych białek wos2 i cis-trans izomerazy peptydylo-prolilowej. Białko wos2 jest odpowiedzialne za fałdowanie białek, wiąże się z chaperonami i HSP90 (Muñoz i in. 1999), podczas gdy cyklofiliny

i izomerazy cis-trans peptydylo-prolilowe są to chaperony, które mogą brać udział w sygnalizacji komórkowej (Wang i Heitman 2005).

W próbach z dodatkiem λ-cyhalotryny i deltametryny wykazano obecność 31-O-demetylo-FK506 FkbM oraz białka wiążącego FK506 (FKBP), które odpowiadają za fałdowanie białek i sygnalizację komórkową (Tong i Jiang 2015). W 48-godzinnych hodowlach deltametryna indukowała wytwarzanie białka rybosomalnego 60S L22, a λ-cyhalotryna - białka rybosomalnego z rodziny L22e. Uzyskane wyniki sugerują, że pyretroidy zaburzają prawidłowe fałdowanie białek.

IV.7. Badanie stresu oksydacyjnego u grzyba entomopatogennego *B. bassiana* pod wpływem pyretroidów

W celu sprawdzenia, czy pyretroidy wywołują stres oksydacyjny u grzyba entomopatogennego *B. bassiana* oznaczono poziom reaktywnych form tlenu (RFT), pojawiających się w komórkach grzybów traktowanych tymi substancjami. Badanie to prowadzono dwuetapowo – na początku określono wpływ pyretroidów w stężeniu 100 mg L^{-1} w hodowlach 24-, 36- i 48-godzinnych (wyniki opisano w pracy P2 (Litwin i in. 2021)), a następnie sprawdzono, czy substancje te w niskim stężeniu (5 mg L^{-1}) również są w stanie wywołać stres oksydacyjny u *B. bassiana* (P3) (Litwin i in. 2023). Wykazano, że α-cypermetryna w stężeniu 100 mg L^{-1} powoduje wzrost ilości anionorodnika ponadtlenkowego (O_2•-) po 24 godzinach inkubacji. W tym punkcie czasowym nie stwierdzono obecności nadtlenku wodoru (H_2O_2). Wszystkie badane pyretroidy w stężeniu 100 mg L^{-1} powodowały istotny wzrost O_2•- w 36- i 48-godzinnych hodowlach oraz wzrost H_2O_2 w hodowlach 48-godzinnych. Zaobserwowany stres oksydacyjny był najprawdopodobniej spowodowany akumulacją pyretroidów w grzybni *B. bassiana*. Wzrost ilości wolnych rodników - O_2•- i tlenku azotu (NO•) zaobserwowano także, gdy badane pyretroidy dodawano do hodowli w stężeniu wyjściowym 5 mg L^{-1}. Obecność H_2O_2 wykazano dopiero w 168-godzinnych hodowlach.

W przeprowadzonych badaniach oznaczono także aktywność enzymów antyoksydacyjnych. Analizy proteomiczne wykazały obecność katalazy (CAT) i dysmutazy ponadtlenkowej (SOD) w grzybni *B. bassiana*. Stwierdzono również wpływ pyretroidów na wytwarzanie tych białek – w próbach z dodatkiem λ-cyhalotryny odnotowano obniżenie produkcji CAT i SOD. Obecność deltametryny hamowała wytwarzanie SOD w 48- i 168-godzinnych hodowlach i indukowała produkcję CAT w hodowlach 168-godzinnych. W literaturze naukowej dostępne są doniesienia na temat wpływu wysokich stężeń deltametryny na aktywność CAT i SOD

u *B. bassiana* (Forlani i in. 2014). W przeprowadzonych badaniach określono wpływ niskiego stężenia pyretroidów (5 mg L^{-1}) na powstawanie stresu oksydacyjnego u tego grzyba entomopatogennego. Odnotowano jedynie wzrost aktywności SOD w hodowlach 168-godzinnych z dodatkiem deltametryny. Chociaż badania proteomiczne sugerowały wpływ pyretroidów na produkcję CAT, nie odnotowano zmian w jej aktywności w obecności tych substancji.

Uzyskane wyniki łączą się z obserwacjami dotyczącymi poziomu ROS w komórkach *B. bassiana.* W drugim etapie badań zaobserwowano obecność nadtlenku wodoru dopiero po 168 godzinach hodowli. Zaobserwowany wzrost aktywności SOD w hodowlach z dodatkiem deltametryny pokrywa się ze spadkiem zawartości O_2•-, przekształcanym przez SOD do H_2O_2 który pojawia się dopiero po 168 godzinach inkubacji.

Przeprowadzone analizy proteomiczne ujawniły obecność peroksyredoksyny, enzymu zdolnego do usuwania szerokiego spektrum ROS (m. in. H_2O_2 i NO•). Wzrost zawartości NO•, zaobserwowany w próbach z dodatkiem α-cypermetryny, najprawdopodobniej przyczynił się do nadprodukcji flawohemoproteiny zaangażowanej w przekształcenie tlenku azotu do azotanu. W obecności α-cypermetryny odnotowano również pojawienie się S-transferazy glutationowej, odpowiedzialnej za metabolizm nadtlenków.

Uzyskane w dysertacji wyniki wyraźnie wskazują, że obecność pyretroidów wywołała stres oksydacyjny u grzyba entomopatogennego *B. bassiana*. Największą odpowiedź antyoksydacyjną zaobserwowano w obecności deltametryny, która indukowała wytwarzanie O_2•- i NO•, powodowała wzrost aktywności dysmutazy ponadtlenkowej oraz powodowała wzrost produkcji białek szoku cieplnego, uważanych za markery stresu.

Nasze wyniki potwierdziły szkodliwość pyretroidów na badany organizm, co jest zgodne z dotychczasowymi obserwacjami dotyczącymi występowania stresu oksydacyjnego u ryb i szczurów w obecności pyretroidów (Kale i in. 1999; Yang i in. 2020).

V. Wnioski i stwierdzenia końcowe

Uzyskane w dysertacji wyniki umożliwiły wyciągnięcie następujących wniosków końcowych:

1. Pyretroidy w stężeniu 50 mg L^{-1} hamują wzrost i zdolność do zarodnikowania *B. bassiana*. Badane substancje nie wywierają natomiast negatywnego wpływu na aktywność metaboliczną ani nie powodują zmian morfologicznych grzybni tego grzyba entomopatogennego. W obecności α-cypermetryny w stężeniu 5 mg L^{-1} zaobserwowano nadprodukcję białek biorących udział w organizacji cytoszkieletu aktynowego oraz enzymów szlaków metabolicznych, co może być mechanizmem obronnym *B. bassiana* umożliwiającym przetrwanie w niesprzyjających warunkach.
2. Pyretroidy powodują spadek zawartości ergosterolu, wzrost stosunku PC/PE oraz spadek zawartości pierwiastków (sodu, potasu, magnezu i wapnia) w komórkach *B. bassiana*. Przedstawione wyniki wskazują na wzrost płynności i przepuszczalności błon w obecności pyretroidów. Niniejsza obserwacja została również potwierdzona testem z jodkiem propidu.
3. Wzrost przepuszczalności błony komórkowej ułatwia wnikanie pyretroidów do wnętrza komórek *B. bassiana* i prowadzi do ich akumulacji. Zależność tą potwierdzono dla pyretroidów dodawanych w niskim jak i w wysokim stężeniu 100 mg L^{-1}.
4. Zakumulowane pyretroidy wpływają na profil fosfolipidowy *B. bassiana,* na produkcję acyloglicerolі oraz na aktywność wewnątrzkomórkowej fosfolipazy C. Zaobserwowany podwyższony stosunek PC/PE wskazuje na zwiększenie płynności błony komórkowej, natomiast wzrost produkcji PI – na wzmożoną syntezę lipidów. Zmiany w ilości lipidów neutralnych oraz aktywności fosfolipazy C, uwidaczniają jak istotny jest wpływ wywoływany przez pyretroidy na *B. bassiana*, ponieważ acyloglicerole biorą udział w mechanizmach niezbędnych do przetrwania grzybów w środowisku naturalnym, natomiast fosfolipaza jest ważnym składnikiem metabolizmu grzybów entomopatogennych.
5. Jednym z mechanizmów oporności *B. bassiana* na pyretroidy jest nadprodukcja białek odpowiedzialnych za wzrost strzępkowy i syntezę ściany komórkowej, co umożliwia naprawę uszkodzonych ścian komórkowych.
6. Uzyskane wyniki wskazują na wpływ pyretroidów na potencjał infekcyjny *B. bassiana*. Obecność α-cypermetryny i deltametryny indukowała produkcję różnych rodzajów

proteaz. Wykazano także, że pyretroidy wpływały na aktywność proteo-, lipo- i chitynolityczną tego grzyba entomopatogennego.

7. Badany drobnoustrój w odpowiedzi na obecność pyretroidów produkuje białka chroniące go przed konsekwencjami wpływu tych substancji. W obecności α-cypermetryny zaobserwowano nadprodukcję białek zaangażowanych w metabolizm związków azotowych. Enzymy te biorą udział w ochronie organizmów przed toksycznym wpływem związków azotowych podczas stresu nitrozacyjnego oraz prawdopodobnie przekształcają obecną w cypermetrynie grupę cyjankową. Dodatek α-cypermetryny powodował także nadprodukcję białek biorących udział w organizacji cytoszkieletu aktynowego, co może tłumaczyć brak zmian morfologicznych w strzępkach tego grzyba. W obecności deltametryny wykazano wzrost produkcji dehydrogenazy alkoholowej odpowiadającej za metabolizm różnych substancji toksycznych.

   Przeprowadzone analizy proteomiczne *B. bassiana* wykazały, że zakumulowane pyretroidy wpływają także na enzymy zaangażowane w główne szlaki metaboliczne oraz zaburzają działanie mitochondriów, odpowiedzialnych za produkcję ATP.

8. Uzyskane wyniki wskazują, że obecność pyretroidów wywołuje stres oksydacyjny u grzyba entomopatogennego *B. bassiana*. Pyretroidy w stężeniu 100 mg L^{-1} indukują wytwarzanie anionorodnika ponadtlenkowego (O_2•-) oraz nadtlenku wodoru (H_2O_2), natomiast w stężeniu 5 mg L^{-1} produkcję tlenku azotu (NO•). Obecność α-cypermetryny powoduje wzrost produkcji flawohemoproteiny oraz S-transferazy glutationowej, natomiast obecność deltametryny - nadprodukcję białek szoku cieplnego a także wzrost aktywności dysmutazy ponadtlenkowej.

VI. Streszczenie

Pyretroidy to syntetyczne insektycydy, których powszechne użycie prowadzi do zanieczyszczenia środowiska oraz wywiera negatywny wpływ na zdrowie organizmów żywych, w tym ludzi. Alternatywą dla chemicznych środków owadobójczych są grzyby entomopatogenne, stosowane do produkcji przyjaznych dla środowiska biopreparatów. Ponieważ pyretroidy i grzyby entomopatogenne stosuje się wymiennie w biokontroli, mikroorganizmy te są narażone na kontakt z pozostałościami tych substancji, gromadzącymi się w glebie, wodach powierzchniowych oraz osadach. Przewodnim celem niniejszej pracy doktorskiej było określenie mechanizmów oporności grzyba entomopatogennego *B. bassiana* na insektycydy z grupy pyretroidów.

W artykule przeglądowym P1 (Litwin i in. 2020) przedstawiono charakterystykę grzybów entomopatogennych, opisano proces infekcyjny, zdolności tych mikroorganizmów do usuwania toksycznych zanieczyszczeń oraz możliwości dodatkowego i niekonwencjonalnego ich wykorzystania. Wyniki przeprowadzonych badań przedstawiono w publikacjach eksperymentalnych P2 (Litwin i in. 2021) oraz P3 (Litwin i in. 2023).

Określono wpływ pyretroidów na wzrost, zarodnikowanie i aktywność metaboliczną *B. bassiana*. Wykazano, że badane substancje w stężeniach 50 i 100 mg L^{-1} powodują istotny spadek biomasy grzybni oraz liczby blastospor. W obecności pyretroidów nie stwierdzono zmian morfologicznych grzybni ani zaburzeń aktywności metabolicznej tego grzyba entomopatogennego, co może wynikać z nadprodukcji białek biorących udział w organizacji cytoszkieletu aktynowego oraz enzymów szlaków metabolicznych wykazanych w obecności α-cypermetryny w stężeniu 5 mg L^{-1}.

W dysertacji, po raz pierwszy wykazano, że pyretroidy akumulują się w grzybni *B. bassiana* a także określono zmiany adaptacyjne zachodzące w odpowiedzi na akumulację. Zaobserwowano wypływ metali z komórek grzyba, najprawdopodobniej będący skutkiem wzrostu przepuszczalności błon komórkowych. Ponadto, określono wpływ zakumulowanych substancji na zawartość ergosterolu, uważanego za marker odpowiedzi komórek na substancje toksyczne. W zależności od zastosowanego stężenia oraz użytej substancji następował wzrost lub spadek ilości ergosterolu w komórkach *B. bassiana*. Wykazano znaczne zahamowanie produkcji metabolitu wtórnego oosporeiny w 36-godzinnych hodowlach w obecności pyretroidów. Stwierdzono wpływ pyretroidów na aktywność wewnątrzkomórkowej fosfolipazy C, zaangażowanej w metabolizm komórkowy grzybów entomopatogennych. Zaobserwowano spadek jej aktywności w hodowlach 48-godzinnych oraz w obecności

α-cypermetryny i deltametryny w hodowlach 168-godzinnych. Dodatek λ-cyhalotryny w hodowlach 120-godzinnych powodował wzrost aktywności tego enzymu.

W przeprowadzonych badaniach potwierdzono wpływ pyretroidów na profil fosfolipidowy oraz zawartość neutralnych lipidów w komórkach *B. bassiana.* Fosfolipidy biorą udział w metabolizmie podstawowym oraz odpowiadają za kondycję błon komórkowych, acyloglicerole natomiast są ważnym elementem szlaku biosyntezy fosfolipidów, jak również zapobiegają powstawaniu reaktywnych form tlenu. W obecności pyretroidów zaobserwowano wzrost stosunku fosfatydylocholiny do fosfatydyloetanoloaminy, co sugeruje, że badane związki powodują zwiększenie płynności błon komórkowych. Wykazano, że wpływ pyretroidów na zawartość acylogliceroli zależy od zastosowanego stężenia tych substancji.

W zewnątrzkomórkowym proteomie *B. bassiana* potwierdzono obecność białek zaangażowanych w proces infekcyjny oraz określono wpływ pyretroidów na ich aktywność. Stwierdzono, że wpływ pyretroidów na aktywność enzymów proteo-, lipo- i chitynolitycznych różni się w zależności od użytej substancji oraz od czasu inkubacji. Wykazano również wzrost aktywności lakazy w hodowlach z dodatkiem pyretroidów.

Stwierdzono, że w obecności deltametryny następowała nadprodukcja antygenowego białka podobnego do taumatyny, natomiast w obecności λ-cyhalotryny i α-cypermetryny – nadprodukcja białek odpowiedzialnych za rozwój strzępek i ściany komórkowej.

W proteomie wewnątrzkomórkowym *B. bassiana* zidentyfikowano białka, których produkcja była indukowana obecnością pyretroidów, wykazano wpływ tych substancji na produkcję białek metabolizmu podstawowego, białek odpowiedzi na stres oraz białek opiekuńczych.

Wykazano, że pyretroidy wywołują stres oksydacyjny u grzyba entomopatogennego *B. bassiana* i zaobserwowano wzrost zawartości wolnych rodników, nadprodukcję flawohemoproteiny oraz S-transferazy glutationowej a także zmiany w produkcji katalazy (CAT) i dysmutazy ponadtlenkowej (SOD). Wykazano także wzrost aktywności SOD w hodowlach 168-godzinnych z dodatkiem deltametryny.

Badania przeprowadzone w ramach realizacji pracy doktorskiej dostarczyły dowodów na zdolność pyretroidów do akumulacji w grzybni entomopatogennego grzyba *B. bassiana*. Wykazano, że zakumulowane pyretroidy, nawet w stosunkowo niskich stężeniach, wywierają negatywny wpływ na kondycję badanego mikroorganizmu a także przedstawiono możliwe mechanizmy oporności *B. bassiana* na przebadane związki toksyczne.

VII. Abstract

Pyrethroids are synthetic insecticides, the widespread use of which leads to environmental pollution and has a negative impact on the health of living organisms, including humans. An alternative to chemical insecticides are entomopathogenic fungi, used to produce environmentally friendly biopreparations. Since pyrethroids and entomopathogenic fungi are used interchangeably in biocontrol, the microorganisms are exposed to contact with residues of these substances that accumulate in soil, surface water and sediment. The main objective of this doctoral thesis was to determine the mechanisms of resistance of the entomopathogenic fungus *B. bassiana* to pyrethroid insecticides.

The review article P1 (Litwin et al. 2020) presents the characteristics of entomopathogenic fungi, describes the infectious process, the ability of these microorganisms to remove toxic impurities and the possibilities of their additional and unconventional use. The results of the conducted research have been presented in experimental publications P2 (Litwin et al. 2021) and P3 (Litwin et al. 2023).

In the study the effect of pyrethroids on the growth, sporulation and metabolic activity of *B. bassiana* was determined. It was shown that the tested substances at concentrations of 50 and 100 mg L^{-1} cause a significant decrease in mycelium biomass and the number of blastospores. In the presence of pyrethroids, no morphological changes in the mycelium or disturbances in the metabolic activity of this entomopathogenic fungus were observed, which may result from the overproduction of proteins involved in the organization of the actin cytoskeleton and enzymes of metabolic pathways demonstrated in the presence of α-cypermethrin at a concentration of 5 mg L^{-1}.

It was shown for the first time that pyrethroids accumulate in the mycelium of *B. bassiana.* Also the adaptive changes that occured in response to accumulation were determined. The efflux of metals from the fungal cells was observed, most likely due to an increase in the permeability of the cell membranes. The effect of accumulated substances on the content of ergosterol, considered a marker of cell response to toxic substances, was determined. Depending on the concentration and the substance used, the level of ergosterol in *B. bassiana* cells increased or decreased.

Significant inhibition of oosporein secondary metabolite production was demonstrated in 36-hour cultures in the presence of pyrethroids. It was found that pyrethroids had an influence on the activity of intracellular phospholipase C, involved in the cellular metabolism of entomopathogenic fungi. A decrease in its activity was observed in 48-hour cultures and

in the presence of α-cypermethrin and deltamethrin in 168-hour cultures. The addition of λ-cyhalothrin in 120-hour cultures increased the activity of this enzyme.

The conducted studies confirmed the influence of pyrethroids on the phospholipid profile and the content of neutral lipids in *B. bassiana* cells. Phospholipids are involved in basic metabolism and are responsible for the condition of cell membranes, while acylglycerols are an important element of the phospholipid biosynthesis pathway and prevent the formation of reactive oxygen species. In the presence of pyrethroids, an increase in the ratio of phosphatidylcholine to phosphatidylethanolamine was observed, which suggests that the tested compounds enhance the fluidity of cell membranes. It was shown that the effect of pyrethroids on the content of acylglycerols depends on the concentration of these substances used.

The presence of proteins involved in the infection process was confirmed in the extracellular proteome of *B. bassiana*, and the influence of pyrethroids on their activity was determined.

It was found that the effect of pyrethroids on the activity of proteo-, lipo- and chitinolytic enzymes differed depending on the substance used and the incubation time. An increase in laccase activity was also demonstrated in cultures supplemented with pyrethroids.

It was observed that in the presence of deltamethrin there was an overproduction of antigenic thaumatin-like protein, while in the presence of λ-cyhalothrin and α-cypermethrin - overproduction of proteins responsible for the hyphae development and the cell wall was noted.

In the intracellular proteome of *B. bassiana*, proteins whose production was induced by the presence of pyrethroids were identified, and the effect of these substances on the production of basic metabolism proteins, stress response proteins and chaperone proteins were demonstrated.

Pyrethroids were shown to induce oxidative stress in the entomopathogenic fungus *B. bassiana*. Also an increase in the reactive oxygen species content, overproduction of flavohemoprotein and glutathione S-transferase, and changes in the production of catalase (CAT) and superoxide dismutase (SOD) were observed. An increase in SOD activity was also demonstrated in 168-hour cultures with the addition of deltamethrin.

Research conducted as part of the doctoral thesis provide evidence of the ability of pyrethroids to accumulate in the mycelium of the entomopathogenic fungus *B. bassiana*. Accumulated pyrethroids, even at relatively low concentrations, were shown to exert a negative impact on the condition of the tested microorganism. Possible mechanisms of resistance of *B. bassiana* to the tested toxic compounds were also presented.

VIII. Całkowity dorobek naukowy

VIII.1. Publikacje

1. Nowak M., Soboń A., **Litwin A.**, Różalska S. 2019. 4-n-nonylphenol degradation by the genus *Metarhizium* with cytochrome P450 involvement. *Chemosphere.* doi: 10.1016/j.chemosphere.2018.12.114.

   IF_{2021} = 8.943; $IF_{5\text{-letni}}$ = 8.520; punkty MEiN = 140; 16 cytowań

2. **Litwin A.**, Nowak M., Różalska S. 2020. Entomopathogenic fungi: unconventional applications. *Reviews in Environmental Science and Bio/Technology*. https://doi.org/10.1007/s11157-020-09525-1

   IF_{2021} = 14.284; $IF_{5\text{-letni}}$ = 12.751; punkty MEiN = 140; 48 cytowań

3. **Litwin A**., Bernat P., Nowak M., Słaba M., Różalska S. 2021. Lipidomic response of the entomopathogenic fungus *Beauveria bassiana* to pyrethroids. *Scientific Reports*. https://doi.org/10.1038/s41598-021-00702-y

   IF_{2021} = 4.997; $IF_{5\text{-letni}}$ = 5.516; punkty MEiN = 140; 3 cytowania

4. **Litwin A.**, Mironenka J., Bernat P., Soboń A., Różalska S. 2023. Accumulation of pyrethroids induces changes in metabolism of the entomopathogenic fungus *Beauveria bassiana*—Proteomic and lipidomic background. *Ecotoxicology and Environmental Safety*. https://doi.org/10.1016/j.ecoenv.2022.114418

   IF_{2021} = 7.129; $IF_{5\text{-letni}}$ = 7.284; punkty MEiN = 100; brak cytowań

Sumaryczny IF_{2021} = 35.353
Sumaryczny $IF_{5\text{-letni}}$ = 34.071
Łączna liczba punktów MEiN = 520
Łączna liczba cytowań = 67
H Index = 3

Podane wartości IF, punktacja MEiN oraz liczba cytowań są zgodne z listą obowiązującą w 2023 roku.
Wartości IF – baza Journal Citation Reports™, stan na dzień 26.01.2023 r.
Liczba cytowań – baza Web of Science, stan na dzień 26.01.2023 r.

VIII.2. Doniesienia konferencyjne

VIII.2.1. Doniesienia konferencyjne w języku polskim

1. **Litwin A.,** Różalska S. 2021. *Określenie wpływu pyretroidów na wzrost oraz równowagę jonową grzyba entomopatogennego Beauveria bassiana.* III Ogólnopolska Przyrodnicza Konferencja Naukowa „Mater naturae" - prezentacja multimedialna online.
2. **Litwin A.,** Różalska S., 2021 *Wpływ pyretroidów na aktywność proteolityczną grzyba entomopatogennego Beauveria bassiana.* II Ogólnopolska Konferencja: Wyzwania Ochrony Środowiska - wystąpienie ustne online
3. **Litwin A.,** Nykiel-Szymańska J., Bernat P., Różalska S. 2019. *Badanie wpływu pyretroidów na żywotność i osłony komórkowe B. bassiana*. III Konferencja Doktorantów Nauk Przyrodniczych – wystąpienie ustne
4. **Litwin A.,** Nowak M., Różalska S. 2018. *Wpływ fosforoorganicznego insektycydu Diazinon na wzrost i żywotność grzybów entomopatogennych z rodzaju Metarhizium.* II Sesja Młodych Mikrobiologów Środowiska Łódzkiego – poster
5. **Litwin A.,** Nowak M., Różalska S. 2018. *Badanie żywotności grzybów entomopatogennych w obecności insektycydów.* IV Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen – poster
6. **Litwin A.,** Nowak M., Różalska S. 2018. *Wpływ insektycydów z grupy pyretroidów na żywotność grzybów entomopatogennych.* Ogólnopolska Konferencja Naukowa dla Doktorantów – wystąpienie ustne
7. **Litwin A.**, Nowak M., Różalska S. 2017. *Wpływ syntetycznych insektycydów oraz substancji aktywnych na wzrost i żywotność grzybów entomopatogennych z rodzaju Isaria.* IV edycja Konferencji Mała Wielka Nauka – wystąpienie ustne
8. Nowak M**., Litwin A.,** Soboń A., Różalska S. 2018. *Biodegradacja ksenoestrogenu (4-n-nonylofenolu) przez grzyby strzępkowe należące do rodzaju Metarhizium.* IV edycja Konferencji Mała Wielka Nauka – wystąpienie ustne
9. **Litwin A.,** Nowak M., Tkaczuk C., Różalska S. 2017. *Wpływ insektycydu Decis na aktywność metaboliczną grzybów entomopatogennych.* Metagenomy różnych środowisk – poster
10. Nowak M., **Litwin A.,** Różalska S. 2017. *Eliminacja 4-n-nonylofenolu przez Metarhizium sp.* Sesja Młodych Mikrobiologów Środowiska Łódzkiego – poster
11. Krupiński M., **Litwin A**., Długoński J. 2016. *Eliminacja i detoksykacja triklosanu przez szczep Pseudomonas putida.* XXVIII Zjazd Polskiego Towarzystwa Mikrobiologów – poster
12. **Litwin A**., Krupiński M., Długoński J. 2015. *Alkilofenole jako modulatory hormonalne, przykłady, charakterystyka oraz możliwość ich degradacji*. III Edycja Konferencji „Mikrobiologia w medycynie, przemyśle i ochronie środowiska – poster
13. Góralczyk A., **Litwin A**., Wojtania D., Jasińska A., Długoński J. 2015. *Zastosowanie technik chromatograficznych w badaniach proteomicznych grzybów strzępkowych.* IV Międzyuczelniane Sympozjum Biotechnologiczne im Prof. K.W. Szewczyka – poster

VIII.2.2. Doniesienia konferencyjne w języku angielskim

1. **Litwin A.,** Różalska S. 2021. *Effect of pyrethroids on the lipolytic activity of Beauveria bassiana.* 6th Edition National Scientific Conference "Promovendi"- wystąpienie ustne online
2. **Litwin A.,** Różalska S. 2021. *Beauveria bassiana oxidative stress proteins induced by the presence of pyrethroids.* 6th Edition National Scientific Conference "Promovendi" - wystąpienie ustne online
3. **Litwin A.,** Różalska S. 2021. *Effect of pyrethroids on the chitinolytic activity of Beauveria bassiana.* Biopotencjał 2021 Warszawa - wystąpienie ustne
4. **Litwin A.,** Różalska S. 2021. *Comparison of pesticides effect on three entomopathogenic fungal strains of the genus Isaria.* National Scientific Conference „Knowledge – Key to Success 2021" 5th edition - wystąpienie ustne online
5. **Litwin A.,** Różalska S. 2021. *The influence of heavy metals on entomopathogenic fungi.* National Scientific Conference „Knowledge – Key to Success 2021" 5th edition - wystąpienie ustne online
6. **Litwin A.,** Różalska S. 2020. *Changes in the extracellular proteome of the entomopathogenic B. bassiana fungus treated with insecticides from the pyrethroid group.* 2nd Virtual Festival of Life and Earth Sciences - prezentacja multimedialna, konferencja w trybie online
7. **Litwin A.,** Różalska S. 2020. *Biosynthesis of metal nanoparticles by entomopathogenic fungi.* 2nd Virtual Festival of Life and Earth Sciences - prezentacja multimedialna, konferencja w trybie online
8. **Litwin A.,** Różalska S. 2020. *Influence of toxic substances on entomopathogenic fungi.* 2nd Virtual Festival of Life and Earth Sciences - prezentacja multimedialna, konferencja w trybie online
9. **Litwin A.,** Filipowska K., Różalska S. 2020. *Determining the effect of pyrethroids insecticides on the ability of entomopathogenic fungi to produce lytic enzymes.* 1st Virtual Festival of Life and Earth Sciences - prezentacja multimedialna – konferencja w trybie online
10. **Litwin A.,** Krzyżanowicz K., Różalska S. 2020. *Determining the effect of insecticides on the viability of yeasts and entomopathogenic fungi.* 1st Virtual Festival of Life and Earth Sciences - prezentacja multimedialna – konferencja w trybie online
11. **Litwin A.,** Różalska S. 2020. *Changes in the ergosterol content in entomopathogenic fungus Beauveria bassiana treated with insecticides from the pyrethroid group.* National Scientific Conference „Science and Young Researchers" IV edition – prezentacja multimedialna – konferencja w trybie online
12. **Litwin A.,** Różalska S. 2020. *Pyrethroids effect on the level of reactive oxygen species in the entomopathogenic fungus Beauveria bassiana.* National Scientific Conference „Science and Young Researchers" IV edition – prezentacja multimedialna – konferencja w trybie online
13. **Litwin A.,** Różalska S. 2020. *Prospects of using entomopathogenic fungi to remove residues of harmful substances from the natural environment.* National Scientific Conference "Knowledge – Key to Success" IV edition – wystąpienie ustne
14. **Litwin A.,** Nykiel-Szymańska J., Tkaczuk C., Słaba M., Różalska S. 2019. *Studies of the relationship between insecticides and entomopathogenic fungi – the*

possibility of their co-application. 7th Central European Congress of Life Sciences – poster

15. **Litwin A.,** Bernat P., Tkaczuk C., Różalska S. 2019. *Effect of pyrethroids on the phospholipid profile of Beauveria bassiana*. 18th Congress of European Mycologists – poster
16. **Litwin A.,** Słaba M., Różalska S. 2019. *The study of the accumulation of insecticides and the impact on cell permeability in entomopathogenic fungus Beauveria bassiana*. Intercollegiate Biotechnology Symposium „Symbioza" – wystąpienie ustne
17. **Litwin A.,** Filipowska K., Różalska S. 2019. *Effect of pyrethroids on the phospholipid profile of Beauveria bassiana*. MycoRise Up! Młodzi w mykologii – wystąpienie ustne
18. **Litwin A.,** Różalska S. 2019. *The study of the infectious properties of entomopathogenic fungi using molecular methods*. National Scientific Conference „Knowledge – Key to Success 2019" - wystąpienie ustne
19. **Litwin A.,** Justyna Nykiel – Szymańska J., Różalska S. 2018. *The influence of pyrethroid insecticides on the production of antioxidative enzymes in entomopathogenic fungi*. Ogólnopolska Konferencja Naukowa „Nauka, Badania, Rozwój" – II Edycja - wystąpienie ustne
20. **Litwin A.,** Złoty M., Różalska S. 2018. *The influence of essential oils – thyme and geranium – on the growth and viability of entomopathogenic fungi from the genus Metarhizium.* XII Copernican International Young Scientists Conference – poster
21. **Litwin A.,** Nowak M., Różalska S. 2018. *Comparison of the impact of pyrethroids and neonicotinoids on the growth and viability of the entomopathogenic fungus Metarhizium robertsii.* XII Copernican International Young Scientists Conference – wystąpienie ustne
22. Nowak M., **Litwin A**., Różalska S. 2018. *Effect of neonicotinoids (acetamiprid and imidacloprid) on the growth and viability of entomopathogenic fungi from the genus Metarhizium, Isaria and Beauveria.* XII Copernican International Young Scientists Conference – poster
23. Nowak M., **Litwin A.,** Różalska S. 2018. *The role of cytochrome p-450 in the 4-n-NP degradation process by entomopathogenic fungus Metarhizium robertsii.* Biotechnology – Research and Industrial Applications Conference – poster
24. Mrozińska J., Złoty M., **Litwin A.,** Różalska S. 2018. *The influence of essential oils on the viability of entomopathogenic fungi*. VII Międzyuczelniane Sympozjum Biotechnologiczne „Symbioza" imienia profesora Krzysztofa W. Szewczyka – poster
25. **Litwin A.,** Barańska J., Złoty M., Różalska S. 2017. *The effect of insecticide Spintor 240 SC on entomopathogenic fungi.* Mikrobiologia w Ochronie Zdrowia i Środowiska – poster
26. Różalska S., **Litwin A.,** Nowak M. 2017. *Influence of acaricide Ortus on the metabolic activity of entomopathogenic fungi.* Mikrobiologia w Ochronie Zdrowia i Środowiska – poster
27. **Litwin A.,** Soboń A. Różalska S. 2017. *Cytosolic fraction proteins of Metarhizium robertsii IM2358 involved in the degradation of 4-n-nonylphenol.* 6th Central European Congress of Life Science – poster

28. **Litwin A.,** Barańska J., Złoty M., Różalska S. 2017. *The effect of insecticides on entomopathogenic fungi*. Wrocławska Konferencja Studentów Nauk Technicznych i Ścisłych PUZZEL – poster
29. **Litwin A.,** Różalska S. 2017. *Interactions of essential oils and entomopathogenic fungi.* Wrocławska Konferencja Studentów Nauk Technicznych i Ścisłych PUZZEL – wystąpienie ustne
30. **Litwin A.,** Różalska S. 2016. *Practical application of entomopathogenic fungi.* Academic Students' Society of Biotechnology – poster
31. **Litwin A.,** Krupiński M., Różalska S. 2016. *Biodegradation and detoxification of triclosan.* Academic Students' Society of Biotechnology – wystąpienie ustne
32. Zawadzka K., **Litwin A**., Felczak A., Lisowska K. 2016. *Degradation of chosen N-heterocyclic compounds with pharmacological activity by filamentous fungi.* 17th European Congress on Biotechnology – poster
33. **Litwin A.**, Różalska S., Długoński J. 2016. *Application of microscopy on studies of oxidative stress in fungi.* Vth International Conference of Biophysics Students – poster
34. **Litwin A**., Skowron M., Strąk D., Krupiński M., Długoński J. 2016. *Evaluation of bacterial and microscopic fungal cultures for their triclosan degradation ability using gas chromatography-mass spectrometry.* V International Conference of Biophysics Students – poster
35. Wojtania D., Rutkowska J., Litwin A., Długoński J. 2014. *Microorganism in dyes biodegradation.* XVI National Academic Seminar of Biotechnology Students & VI International Conference of Biotechnology Students – poster

VIII.3. Aktywność organizacyjna:

1. Udział w organizacji V Ogólnopolskiej Konferencji Doktorantów Nauk o Życiu BioOpen 2019 – 30-31 maja 2019, Łódź.
2. Udział w organizacji IV Ogólnopolskiej Konferencji Doktorantów Nauk o Życiu BioOpen 2018 – 24-25 maja 2018, Łódź.
3. Przeprowadzenie warsztatów pt. „Biotechnologia od kuchni" podczas Ogólnopolskiej Nocy Biologów, 12.01.2018, Łódź.
4. Udział w organizacji stoiska „Ekohydrologia - świat niezwykłych zależności pomiędzy wodą i ekologią", wystawianego przez Katedrę Ekologii Stosowanej, Wydziału Biologii i Ochrony Środowiska UŁ. Festiwal Nauki, Techniki i Sztuki, 21-22.04.2018, Łódź.
5. Udział w organizacji Międzynarodowego Sympozjum „Ecohydrology for the Circular Economy and Nature-Based Solutions towards mitigation/adaptation to Climate Change" – 26-28 września 2017, Łódź.
6. Udział w organizacji IV edycji konferencji naukowej „Mikrobiologia w Ochronie Zdrowia i Środowiska" – MIKROBIOT 2017 – 19-21 września 2017, Łódź.
7. Aktywne uczestnictwo w promocji studiów doktoranckich Wydziału Biologii i Ochrony Środowiska w ramach I Dnia Doktoranta Uniwersytetu Łódzkiego – 22 maja 2017 – Łódź.
8. Udział w organizacji III Ogólnopolskiej Konferencji Doktorantów Nauk o Życiu BioOpen 2017 – 11-12 maja 2017, Łódź.

9. Udział w organizacji warsztatów „Zastosowanie mikroskopii konfokalnej w badaniach mikrobiologicznych, biotechnologicznych, mykologicznych i parazytologicznych” – 3 lutego 2017, Łódź.
10. Praca ze Studenckim Kołem Naukowym Biotechnologiczno – Mikrobiologicznym SKN Bio-Mik działającym przy Katedrze Mikrobiologii Przemysłowej i Biotechnologii, Wydziału Biologii i Ochrony Środowiska UŁ.

VIII.4. Szkolenia

1. Udział w szkoleniu „Współpraca nauki z biznesem. Jak wdrażać i chronić innowacyjne pomysły” Fundacja Rozwoju Przedsiębiorczości. 21 października 2016, Łódź.

IX. Literatura

Abidin AF, Ekowati N, Ratnaningtyas NI (2017) Insecticide compatibility to the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae. Scr Biol 4:273–279

Aouini F, Boussoufa D, Ghribi F, et al (2017) Short term exposure to permethrin alters lipid classes and fatty acid composition of the unfed clam Donax trunculus from Tunisian coast (Southern Mediterranean). Cah Biol Mar 58:163–172. https://doi.org/10.21411/CBM.A.72E9D4EA

Barman A, Gohain D, Bora U, Tamuli R (2018) Phospholipases play multiple cellular roles including growth, stress tolerance, sexual development, and virulence in fungi. Microbiol Res 209:55–69. https://doi.org/10.1016/j.micres.2017.12.012

Behie SW, Bidochka MJ (2014) Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: An additional branch of the soil nitrogen cycle. Appl Environ Microbiol 80:1553–1560. https://doi.org/10.1128/AEM.03338-13

Benito B, Garciadeblás B, Fraile-Escanciano A, Rodríguez-Navarro A (2011) Potassium and sodium uptake systems in fungi. The transporter diversity of Magnaporthe oryzae. Fungal Genet Biol 48:812–822. https://doi.org/10.1016/j.fgb.2011.03.002

Bernat P, Gajewska E, Szewczyk R, et al (2014) Tributyltin (TBT) induces oxidative stress and modifies lipid profile in the filamentous fungus Cunninghamella elegans. Environ Sci Pollut Res 21:4228–4235. https://doi.org/10.1007/s11356-013-2375-5

Bernat P, Nykiel-Szymańska J, Stolarek P, et al (2018) 2,4-dichlorophenoxyacetic acid-induced oxidative stress: Metabolome and membrane modifications in umbelopsis isabellina, a herbicide degrader. PLoS One 13:1–18. https://doi.org/10.1371/journal.pone.0199677

Bhatt P, Huang Y, Zhan H, Chen S (2019) Insight into microbial applications for the biodegradation of pyrethroid insecticides. Front Microbiol 10:1–19. https://doi.org/10.3389/fmicb.2019.01778

Bilgo E, Lovett B, St Leger RJ, et al (2018) Native entomopathogenic Metarhizium spp. from Burkina Faso and their virulence against the malaria vector Anopheles coluzzii and non-target insects. Parasites and Vectors 11:11–16. https://doi.org/10.1186/s13071-018-2796-6

Boysen JM, Saeed N, Wolf T, et al (2021) The peroxiredoxin asp f3 acts as redox sensor in Aspergillus fumigatus. Genes (Basel) 12:668. https://doi.org/10.3390/genes12050668

Breitenbach M, Weber M, Rinnerthaler M, et al (2015) Oxidative stress in fungi: Its function in signal transduction, interaction with plant hosts, and lignocellulose degradation. Biomolecules 5:318–342. https://doi.org/10.3390/biom5020318

Calmes B, Morel-Rouhier M, Bataillé-Simoneau N, et al (2015) Characterization of glutathione transferases involved in the pathogenicity of Alternaria brassicicola. BMC Microbiol 15:1–10. https://doi.org/10.1186/s12866-015-0462-0

Castro T, Mayerhofer J, Enkerli J, et al (2016) Persistence of Brazilian isolates of the entomopathogenic fungi Metarhizium anisopliae and M. robertsii in strawberry crop soil after soil drench application. Agric Ecosyst Environ 233:361–369. https://doi.org/10.1016/j.agee.2016.09.031

Chen X-M, Wang X-Y, Lu W, Zheng X-L (2021) Use of Beauveria bassiana in combination with commercial insecticides to manage Phauda flammans (Walker) (Lepidoptera:

Phaudidae): Testing for compatibility and synergy. J Asia Pac Entomol 24:272–278
Chrustek A, Hołyńska-Iwan I, Dziembowska I, et al (2018) Current research on the safety of pyrethroids used as insecticides. Med 54:1–15. https://doi.org/10.3390/medicina54040061
Cycoń M, Piotrowska-Seget Z (2016) Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: A review. Front Microbiol 7:1–26. https://doi.org/10.3389/fmicb.2016.01463
Dannon HF, Dannon AE, Douro-Kpindou OK, et al (2020) Toward the efficient use of Beauveria bassiana in integrated cotton insect pest management. J Cott Res 3:24. https://doi.org/10.1186/s42397-020-00061-5
Dhawan M, Joshi N (2017) Enzymatic comparison and mortality of Beauveria bassiana against cabbage caterpillar Pieris brassicae LINN. Brazilian J Microbiol 48:522–529. https://doi.org/10.1016/j.bjm.2016.08.004
Djordjevic JT (2010) Role of phospholipases in fungal fitness, pathogenicity, and drug development - lessons from Cryptococcus neoformans. Front Microbiol 1:1–13. https://doi.org/10.3389/fmicb.2010.00125
Donzelli BGG, Krasnoff SB (2016) Molecular Genetics of Secondary Chemistry in Metarhizium Fungi. Adv Genet 94:365–436. https://doi.org/10.1016/bs.adgen.2016.01.005
Ebani VV, Mancianti F (2021) Entomopathogenic fungi and bacteria in a veterinary perspective. Biology (Basel) 10:1–31. https://doi.org/10.3390/biology10060479
Fan J, Yu L, Xu C (2017a) A central role for triacylglycerol in membrane lipid breakdown, fatty acid β-oxidation, and plant survival under extended darkness. Plant Physiol 174:1517–1530. https://doi.org/10.1104/pp.17.00653
Fan Y, Liu X, Keyhani NO, et al (2017b) Regulatory cascade and biological activity of Beauveria bassiana oosporein that limits bacterial growth after host death. Proc Natl Acad Sci U S A 114:E1578–E1586. https://doi.org/10.1073/pnas.1616543114
Feng P, Shang Y, Cen K, Wang C (2015) Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc Natl Acad Sci 112:11365–11370. https://doi.org/10.1073/pnas.1503200112
Forlani L, Juárez MP, Lavarías S, Pedrini N (2014) Toxicological and biochemical response of the entomopathogenic fungus Beauveria bassiana after exposure to deltamethrin. Pest Manag Sci 70:751–756. https://doi.org/10.1002/ps.3583
Gao BJ, Mou YN, Tong SM, et al (2020) Subtilisin-like Pr1 proteases marking the evolution of pathogenicity in a wide-spectrum insect-pathogenic fungus. Virulence 11:365–380. https://doi.org/10.1080/21505594.2020.1749487
Gao Q, Lu Y, Yao H, et al (2016) Phospholipid homeostasis maintains cell polarity, development and virulence in Metarhizium robertsii. Environ Microbiol 18:3976–3990. https://doi.org/10.1111/1462-2920.13408
Gibson DM, Donzelli BGG, Krasnoff SB, Keyhani NO (2014) Discovering the secondary metabolite potential encoded within entomopathogenic fungi. Nat Prod Rep 31:1287–1305. https://doi.org/10.1039/c4np00054d
Gonzalez R, Nicolau F, Peeples TL (2017) Optimization of the 11α-hydroxylation of steroid DHEA by solvent-adapted Beauveria bassiana. Biocatal Biotransformation 35:103–109. https://doi.org/10.1080/10242422.2017.1289183
Hausladen A, Gow AJ, Stamler JS (1998) Nitrosative stress: Metabolic pathway involving the flavohemoglobin. Proc Natl Acad Sci U S A 95:14100–14105. https://doi.org/10.1073/pnas.95.24.14100
Hu Y, Zhang Y, Vinturache A, et al (2020) Effects of environmental pyrethroids exposure on semen quality in reproductive-age men in Shanghai, China,. Chemosphere 245:125580

Huarte-Bonnet C, Juárez MP, Pedrini N (2015) Oxidative stress in entomopathogenic fungi grown on insect-like hydrocarbons. Curr Genet 61:289–297. https://doi.org/10.1007/s00294-014-0452-z
Joshi M, Gaur N, Pandey R (2018) Compatibility of entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae with selective pesticides. J Entomol Zool Stud 6:867–872
Kale M, Rathore N, John S, Bhatnagar D (1999) Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol Lett 105:197–205
Kim H ul, Bharda AV, Moon JC, et al (2021) Microscopic studies on severing properties of actin-binding protein: its potential use in therapeutic treatment of actin-rich inclusions. J Anal Sci Technol 12:10–15. https://doi.org/10.1186/s40543-021-00305-2
Kos K, Celar FA (2013) Sensitivity of the entomopathogenic fungus Beauveria bassiana (Bals.-Criv.) Vuill. to selected herbicides. Pest Manag Sci 69:717–721. https://doi.org/10.1002/ps.3427
Łaniewska-Dunaj M, Jelski W, Szmitkowski M (2013) Dehydrogenaza alkoholowa - Znaczenie fizjologiczne i diagnostyczne. Postepy Hig Med Dosw 67:901–907. https://doi.org/10.5604/17322693.1064330
Lee SJ, Lee MR, Kim S, et al (2018) Genomic Analysis of the Insect-Killing Fungus Beauveria bassiana JEF-007 as a Biopesticide. Sci Rep 8:1–12. https://doi.org/10.1038/s41598-018-30856-1
Li H, Cheng F, Wei Y, et al (2017) Global occurrence of pyrethroid insecticides in sediment and the associated toxicological effects on benthic invertebrates: An overview. J Hazard Mater 324:258–271. https://doi.org/10.1016/j.jhazmat.2016.10.056
Litwin A, Bernat P, Nowak M, et al (2021) Lipidomic response of the entomopathogenic fungus Beauveria bassiana to pyrethroids. Sci Rep 11:1–11. https://doi.org/10.1038/s41598-021-00702-y
Litwin A, Mironenka J, Bernat P, et al (2023) Accumulation of pyrethroids induces changes in metabolism of the entomopathogenic fungus Beauveria bassiana—Proteomic and lipidomic background. Ecotoxicol Environ Saf 249:114418. https://doi.org/10.1016/j.ecoenv.2022.114418
Litwin A, Nowak M, Różalska S (2020) Entomopathogenic fungi: unconventional applications. Rev Environ Sci Biotechnol 19:23–42. https://doi.org/10.1007/s11157-020-09525-1
Liu JJ, Sturrock R, Ekramoddoullah AKM (2010) The superfamily of thaumatin-like proteins: Its origin, evolution, and expression towards biological function. Plant Cell Rep 29:419–436. https://doi.org/10.1007/s00299-010-0826-8
Liu SQ, Meng ZH, Yang JK, et al (2007) Characterizing structural features of cuticle-degrading proteases from fungi by molecular modeling. BMC Struct Biol 7:33. https://doi.org/10.1186/1472-6807-7-33
Lovera A, Belaich M, Villamizar L, et al (2020) Enhanced virulence of Beauveria bassiana against Diatraea saccharalis using a soluble recombinant enzyme with endo- and exochitinase activity. Biol Control 144:104211. https://doi.org/10.1016/j.biocontrol.2020.104211
Lu Z, Deng J, Wang H, et al (2021) Multifunctional role of a fungal pathogen-secreted laccase 2 in evasion of insect immune defense. Environ Microbiol 23:1256–1274. https://doi.org/10.1111/1462-2920.15378
Mantzoukas S, Eliopoulos PA (2020) Endophytic entomopathogenic fungi: A valuable biological control tool against plant pests. Appl Sci 10:360. https://doi.org/10.3390/app10010360

Martínková L, Veselá AB, Rinágelová A, Chmátal M (2015) Cyanide hydratases and cyanide dihydratases: emerging tools in the biodegradation and biodetection of cyanide. Appl Microbiol Biotechnol 99:8875–8882. https://doi.org/10.1007/s00253-015-6899-0
Mascarin GM, Jaronski ST (2016) The production and uses of Beauveria bassiana as a microbial insecticide. World J Microbiol Biotechnol 32:1–26. https://doi.org/10.1007/s11274-016-2131-3
Mc Namara L, Dolan SK, Walsh JMD, et al (2019) Oosporein, an abundant metabolite in Beauveria caledonica, with a feedback induction mechanism and a role in insect virulence. Fungal Biol 123:601–610. https://doi.org/10.1016/j.funbio.2019.01.004
Mondal S, Baksi S, Koris A, Vatai G (2016) Journey of enzymes in entomopathogenic fungi. Pacific Sci Rev A Nat Sci Eng 18:85–99. https://doi.org/10.1016/j.psra.2016.10.001
Morel M, Ngadin AA, Droux M, et al (2009) The fungal glutathione S-transferase system. Evidence of new classes in the wood-degrading basidiomycete Phanerochaete chrysosporium. Cell Mol Life Sci 66:3711–3725. https://doi.org/10.1007/s00018-009-0104-5
Muñoz MJ, Bejarano ER, Daga RR, Jimenez J (1999) The identification of Wos2, a p23 homologue that interacts with Wee1 and Cdc2 in the mitotic control of fission yeasts. Genetics 153:1561–1572. https://doi.org/10.1093/genetics/153.4.1561
Natali de Oliveira C, Manuel Oliveira Janeiro Neves P, Sueki Kawazoe L (2003) Compatibility between the entomopathogenic fungus beauveria bassiana and insecticides used in coffee plantations. Sci Agric 60:663–667
Nolan LM, Harnedy PA, Turner P, et al (2003) The cyanide hydratase enzyme of Fusarium lateritium also has nitrilase activity. FEMS Microbiol Lett 221:161–165. https://doi.org/10.1016/S0378-1097(03)00170-8
Nowak M, Bernat P, Mrozińska J, Rózalska S (2020) Acetamiprid Affects Destruxins Production but Its Accumulation in Metarhizium sp. Spores Increases Infection Ability of Fungi. Toxins (Basel) 12:587. https://doi.org/10.3390/toxins12090587
Nowak M, Soboń A, Litwin A, Różalska S (2019) 4-n-nonylphenol degradation by the genus Metarhizium with cytochrome P450 involvement. Chemosphere 220:324–334. https://doi.org/10.1016/j.chemosphere.2018.12.114
Nykiel-Szymańska J, Różalska S, Bernat P, Słaba M (2019) Assessment of oxidative stress and phospholipids alterations in chloroacetanilides-degrading Trichoderma spp. Ecotoxicol Environ Saf 184:109629
Oide S, Tanaka Y, Watanabe A, Inui M (2019) Carbohydrate-binding property of a cell wall integrity and stress response component (WSC) domain of an alcohol oxidase from the rice blast pathogen Pyricularia oryzae. Enzyme Microb Technol 125:13–20
Palma-Guerrero J, Lopez-Jimenez JA, Pérez-Berná AJ, et al (2010) Membrane fluidity determines sensitivity of filamentous fungi to chitosan. Mol Microbiol 75:1021–1032. https://doi.org/10.1111/j.1365-2958.2009.07039.x
Pedrini N, Rosana C, Juarez M. Patricia (2007) Biochemistry of insect epicuticule degradation by entomopathogenic fungi. Comp Biochem Physiol 146:124–137
Rahmanpour S, Backhouse D, Nonhebel HM (2009) Induced tolerance of sclerotinia sclerotiorum to isothiocyanates and toxic volatiles from brassica species. Plant Pathol 58:479–486. https://doi.org/10.1111/j.1365-3059.2008.02015.x
Ríos-Moreno A, Garrido-Jurado I, Resquín-Romero G, et al (2016) Destruxin A production by Metarhizium brunneum strains during transient endophytic colonisation of Solanum tuberosum. Biocontrol Sci Technol 26:1574–1585. https://doi.org/10.1080/09583157.2016.1223274
Rocha MC, de Godoy KF, Bannitz-Fernandes R, et al (2018) Analyses of the three 1-Cys Peroxiredoxins from Aspergillus fumigatus reveal that cytosolic Prx1 is central to H2O2

metabolism and virulence. Sci Rep 8:1–18. https://doi.org/10.1038/s41598-018-30108-2
Różalska S, Glińska S, Długoński J (2014) Metarhizium robertsii morphological flexibility during nonylphenol removal. Int Biodeterior Biodegrad 95:285–293. https://doi.org/10.1016/j.ibiod.2014.08.002
Różalska S, Soliwoda K, Długoński J (2016) Synthesis of silver nanoparticles from Metarhizium robertsii waste biomass extract after nonylphenol degradation, and their antimicrobial and catalytic potential. RSC Adv 6:21475–21485. https://doi.org/10.1039/c5ra24335a
Santi L, Beys da Silva WO, Berger M, et al (2010) Conidial surface proteins of Metarhizium anisopliae: Source of activities related with toxic effects, host penetration and pathogenesis. Toxicon 55:874–880. https://doi.org/10.1016/j.toxicon.2009.12.012
Skinner M, Parker BL, Kim JS (2014) Role of Entomopathogenic Fungi in Integrated Pest Management. In: Abrol DP (ed) Integrated Pest Management: Current Concepts and Ecological Perspective. Elsevier Inc., pp 169–191
Soboń A, Szewczyk R, Długoński J, Różalska S (2019) A proteomic study of Cunninghamella echinulata recovery during exposure to tributyltin. Environ Sci Pollut Res 26:32545–32558. https://doi.org/10.1007/s11356-019-06416-z
Sotelo-Mundo RR, Lopez-Zavala AA, Garcia-Orozco KD, et al (2007) The lysozyme from insect (Manduca sexta) is a cold-adapted enzyme. Protein Pept Lett 14:774–778
Stolarek P, Różalska S, Bernat P (2019) Lipidomic adaptations of the Metarhizium robertsii strain in response to the presence of butyltin compounds. Biochim Biophys Acta - Biomembr 1861:316–326. https://doi.org/10.1016/j.bbamem.2018.06.007
Suchodolski J, Muraszko J, Bernat P, Krasowska A (2019) A crucial role for ergosterol in plasma membrane composition, localisation, and activity of Cdr1p and H+-ATPase in Candida albicans. Microorganisms 7:1–17. https://doi.org/10.3390/microorganisms7100378
Szewczyk R, Kuśmierska A, Bernat P (2018) Ametryn removal by Metarhizium brunneum: Biodegradation pathway proposal and metabolic background revealed. Chemosphere 190:174–183. https://doi.org/10.1016/j.chemosphere.2017.10.011
Tang T, Wu R, Zhang L, et al (2021) Distribution and partitioning of pyrethroid insecticides in agricultural lands: Critical influencing factors. Environ Int 156:106736. https://doi.org/10.1016/j.envint.2021.106736
Tang W, Wang D, Wang J, et al (2018) Pyrethroid pesticide residues in the global environment: An overview. Chemosphere 191:990–1007. https://doi.org/10.1016/j.chemosphere.2017.10.115
Tisi R, Baldassa S, Belotti F, Martegani E (2002) Phospholipase C is required for glucose-induced calcium influx in budding yeast. FEBS Lett 520:133–138. https://doi.org/10.1016/S0014-5793(02)02806-5
Tong M, Jiang Y (2015) FK506-Binding Proteins and Their Diverse Functions. Curr Mol Pharmacol 9:48–65
Wang H, Peng H, Li W, et al (2021) The Toxins of Beauveria bassiana and the Strategies to Improve Their Virulence to Insects. Front Microbiol 12:1–11. https://doi.org/10.3389/fmicb.2021.705343
Wang J, Chen J, Hu Y, et al (2020) Roles of six Hsp70 genes in virulence, cell wall integrity, antioxidant activity and multiple stress tolerance of Beauveria bassiana. Fungal Genet Biol 144:103437
Wang P, Heitman J (2005) The cyclophilins. Genome Biol 6:226. https://doi.org/10.1186/gb-2005-6-7-226
Xiong Z, Wei Q, Chen H, et al (2006) Microbial transformation of androst-4-ene-3,17-dione by Beauveria bassiana. Steroids 71:979–983.

https://doi.org/10.1016/j.steroids.2006.07.007
Xu L, Ferry N, Wang Z, et al (2013) A proteomic approach to study the mechanism of tolerance to Bt toxins in Ostrinia furnacalis larvae selected for resistance to Cry1Ab. Transgenic Res 22:1155–1166. https://doi.org/10.1007/s11248-013-9718-3
Yang C, Lim W, Song G (2020) Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. Comp Biochem Physiol Part - C Toxicol Pharmacol 234:108758
Zhang X, Zhang T, Ren X, et al (2021) Pyrethroids Toxicity to Male Reproductive System and Offspring as a Function of Oxidative Stress Induction: Rodent Studies. Front Endocrinol (Lausanne) 12:1–12. https://doi.org/10.3389/fendo.2021.656106

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REVIEW PAPER

Entomopathogenic fungi: unconventional applications

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Abstract Entomopathogenic fungi (EPF) are microorganisms that cause fatal diseases of arthropods. The infection process involves several stages that consist of direct contact of the fungus with the surface of the cuticle of the attacked insect. The factors that determine the effectiveness of the infection process include lytic enzymes, secondary metabolites, and adhesins produced by EPF. Because of their high insecticidal effectiveness, these fungi are commonly used as biopesticides in organic farming. As the environment and farmlands are contaminated with many compounds of anthropogenic origin (e.g., pesticides), the effects of these toxic compounds on EPF and the mechanisms that affect their survival in such a toxic environment have been studied in recent years. This review presents information on the capacity of EPF to remove toxic contaminants, including alkylphenols, organotin compounds, synthetic estrogens, pesticides and hydrocarbons. Moreover, these fungi produce numerous secondary metabolites that can be potentially used in medicine or as antimicrobial agents. Despite their huge potential in biocontrol processes, the use of EPF has been underestimated due to a lack of knowledge on their abilities. In our work, we have presented the available data on the possibilities of the additional and unconventional use of these microorganisms.

Keywords Entomopathogenic fungi · Toxic compounds · Nanoparticle biosynthesis · Biodegradation

1 Introduction

Entomopathogenic fungi (EPF) are bioinsecticides with an ability to infect and kill arthropods. Although they are mainly isolated from arthropod carcasses, their natural habitat is soil (Behie and Bidochka 2014). These fungi are categorized in six classes: *Oomycetes*, *Chytridiomycota*, *Microsporidia*, *Entomophtoromycota*, *Basidiomycota*, and the most common *Ascomycota*. The main function of EPF in the environment is the biocontrol of the insect population. The literature data provide information on the infectious properties of EPF, the infectious process (Skinner et al. 2014; Lacey et al. 2015; Mascarin and Jaronski 2016), and the use of these microorganisms in commercial

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preparations as biopesticides (Jaihan et al. 2016; Ríos-Moreno et al. 2016).

In the last few decades, the constantly increasing environmental pollution is a serious concern. Every day substances are being introduced in the environment that have an adverse effect on the balance of the ecosystem and living organisms. Fungi that inhabit the soil are also sensitive to contaminants (e.g., herbicides, insecticides, fungicides and heavy metals) that cause decreased or delayed growth, abnormalities of cellular metabolic pathways, and damage of cellular structures (Różalska et al. 2014; Gola et al. 2018; Szewczyk et al. 2018; Stolarek et al. 2019; Subbanna et al. 2019). Adverse changes in the cells caused by these compounds prevent these microorganisms from playing their proper ecological role. Some fungi have also developed mechanisms that allow them to survive in the presence of such contaminants (Szewczyk et al. 2018; Nowak et al. 2019).

Because of high enzymatic activity, the ability to produce secondary metabolites, and good growth in culture media, EPF are also being tested for use in other fields of biotechnology, e.g., nanoparticle biosynthesis and bioconversion of steroids or flavonoids, which are important from an economic point of view (Różalska et al. 2016; Gonzalez et al. 2017; Yosri et al. 2018; Dou et al. 2019; Kozłowska et al. 2019).

2 Entomopathogenic fungi—short description

Entomopathogenic fungi are parasitic microorganisms with an ability to infect and kill arthropods. They are mainly used as biopesticides in ecologic farming as a safe alternative to toxic chemical insecticides, although some of them are also used in biotechnological processes or in Chinese medicine (Jaihan et al. 2016; Ríos-Moreno et al. 2016; Lovett and Leger 2017).

Entomopathogenic fungi do not form one monophyletic group. Thus far, 12 species of *Oomycetes*, 65 species of *Chytridiomycota*, 339 species of *Microsporidia*, 474 species of *Entomophtoromycota*, 238 species of *Basidiomycota*, and 476 species of *Ascomycota* have been reported (Araújo and Hughes 2016). Species belonging to *Ascomycota* and *Entomophtoromycota* occur most frequently in nature. In the literature, species belonging to *Ascomycota* to the genera *Metarhizium* (*M. anisopliae, M. robertsii, M. brunneum, M. lepidiotae, M. globosum, M. acridum, M. majus, M. flavoviride, M. rileyi, M. pingshaense, M. lepidiotae and M. guizhouense*), *Beauveria (B. bassiana and B. brongniartii), Isaria (I. fumosorosea*—formerly *Paecilomyces fumosoroseus*, *I. farinosa and I. tennuipes), Ophiocordyceps (O. sinensis*—formerly *Cordyceps sinensis*, *O. unilateralis*), *Cordyceps* (*C. militaris*), *Torubiella (T. ratticaudata), Pochonia (P. chlamydosporia)*, *Lecanicillium (L. lecani*—formerly *Verticillium lecanii, L. longisporum)*, *Hirsutella (H. thompsonii, H. nodulosa, H. aphidis)*, and the species *Paecilomyces variotii, Purpureocillium lilacinum* are described (Khan et al. 2012; Tkaczuk et al. 2015; Jaihan et al. 2016.

Among the known EPF, Entomophthorales (e.g., *Furia, Conidiobolus, Entomophaga*, or *Erynia*) show the highest insecticidal activity; however, because of technical difficulties in breeding in laboratory conditions, they are not used as components of biopreparations. The most commonly used EPF are easily cultured saprotrophic fungi belonging to *Ascomycota* (Mascarin and Jaronski 2016). Worldwide, several different biopesticides based on species belonging to the genera *Metarhizium, Beauveria*, *Paecilomyces, Isaria* and *Lecanicillium* have been used. These fungi have a wide spectrum of activity, and they can therefore infect a wide variety of arthropod species (Khan et al. 2012; Castro et al. 2016; Ríos-Moreno et al. 2016).

Entomopathogenic fungi are heterogeneous organisms that play various ecological roles. For example, species of the genus *Metarhizium* and *Beauveria,* which are commonly found in soil, not only control natural arthropod populations but also form complex relationships with plants. They are described as endophytes of plant roots, stems, and leaves (Jaber and Enkerli 2017). It has been shown that *M. robertsii* and *B. bassiana* (but not *L. lecani*) provide plants with nitrogen that is assimilated during the parasitization of insects (Behie and Bidochka 2014), thus supporting plant growth (Ríos-Moreno et al. 2016). *Beauveria bassiana* acts as an endophyte in about 25 plant species, contributing to the control of pests and fungal plant pathogens (McKinnon et al. 2017; Vega 2018). As a fungal endophyte and epiphyte, it colonizes leaves and shoots in addition to plant roots making plants more resistant to insects (Klieber and Reineke 2016; Ramakuwela et al. 2020), and also successfully protects plants from microbial pathogens by

suppressing disease-causing agents or increasing plant defense responses (Moonjely et al. 2016). Similarly, *Lecanicillium* also prevents fungal disease by growing on the surface of leaves, limiting available nutrients and producing antimicrobial compounds in addition to inducing plant responses while colonizing plant roots (Moonjely et al. 2016). Among EPF, *I. fumosorosea* seems not to fall into strong interactions with plants. This is probably due to the fact that *Isaria* spp. are sensitive to chemical substances secreted by plants and belonging to the defense system against plant pathogens (Lacey and Mercadier 1998; Zimmermann 2008). Interestingly, isolates of *I. fumosorosea* are reported to act against the root nematode, *Meloidogyne javanica*, although infection rates were found to be very low (below 40%) (Tigano-Milani et al. 1995). EPF are primarily used in biocontrol of insect pests in the laboratory, greenhouse or in the field.

2.1 The infectious process

Entomopathogenic fungi infect insects by direct penetration of the cuticle. Unlike bacteria or viruses, they do not have to be ingested by an insect (Bilgo et al. 2018). The infection process starts with the adhesion of spores to arthropod shells and has two stages: the first depends on the action of hydrophobic and electrostatic forces and the second requires the activity of enzymes and low-molecular-weight proteins called hydrophobins (Skinner et al. 2014). Spore germination occurs in the presence of carbon and energy sources on the insect's cuticle at sufficient humidity and temperature. The optimum temperature for the growth and germination of EPF is between 20 and 30 °C. Spores can also germinate at temperatures outside this range and this is a characteristic feature of the particular fungal strain (Skinner et al. 2014). Subsequently, appressoria emerge, causing strong mechanical pressure on the cuticle and the production of lytic enzymes (proteo-, lipo- and chitinolytic) that disintegrate the insect's body shells (Skinner et al. 2014; Lacey et al. 2015). After penetrating the arthropod's body cavity (hemocel), the fungal hyphae start to grow. Some EPF can produce blastospores that enter host's hemolymph and produce secondary hyphae that inhabit the host's tissues. At this stage, the fungi produce secondary metabolites that cause paralysis and disrupt the host's physiological processes, mainly its immune responses (Donzelli and Krasnoff 2016). Because of the developing infection, the insect's body is destroyed by both mechanical damage to the internal organs by the developing hyphae and nutrient depletion (Donzelli and Krasnoff 2016; Mascarin and Jaronski 2016; Fan et al. 2017).

As a result of the progressive infection, the insect's body, initially soft, becomes stiff due to fluid absorption by the fungus. Cadavers of insects attacked by fungi of the genus *Beauveria* may initially take on a dark red color. The entire infection process is relatively long and takes approximately 14 days after infection, but first symptoms of infection usually occur about 7 days post infection (or even earlier, depending on fungal species). After killing the insect and using all nutrition, the hyphae of the fungus emerge from the cadaver of the host through holes in its body (mouth hole, anus) and through intersegmental areas. Then, resting or infective spores are produced, which allows the fungus to spread and infect other individuals (Skinner et al. 2014).

2.2 Infectious agents

The pathogenesis caused by EPF requires the involvement of several infectious agents, the most important of which are adhesins, lytic enzymes, and secondary metabolites.

2.2.1 Adhesins

The first stage of the fungal infection process is spore adhesion to the surface of the arthropod's body. In this stage, two types of proteins are produced: hydrophobins (whose layers disintegrate during the sporulation of spores) and adhesins (MAD1 and MAD2), which enable both close adhesion to the insect's cuticle and recognition of the host by the fungal pathogen (Wang and Leger 2007; Greenfield et al. 2014).

2.2.2 Lytic enzymes

Lytic enzymes play the most important role during the process of insect infection by EPF. Their action, structure, and types are relatively well described. Their main role is hydrolysis of the components of the insect cuticle, which allows appressoria to penetrate the outer covers of arthropods. Lytic enzymes are

produced as soon as the spore attaches to the cuticle and begins to form appressorium (Santi et al. 2010).

Lipases are produced first and hydrolyze the lipids and lipoproteins located in the insect epicuticle (outer cuticle) (Pedrini et al. 2007). These enzymes hydrolyze the ester bonds of triacylglycerols, which leads to the release of free fatty acids, diacylglycerols, monoacylglycerols, and glycerol (Silva et al. 2009). Moreover, lipases support the adhesion of germinating spores to insect cuticles by increasing hydrophobic interactions between the fungus and the cuticle surface (Santi et al. 2010). All EPF have been shown to contain these enzymes, most of which have been well described (Supakdamrongkul et al. 2010; Mondal et al. 2016). One of these enzymes is *M. brunneum* (formerly *M. anisopliae* var. *anisopliae*) phospholipase C, which hydrolyzes phosphodiester bonds and is responsible for degrading phospholipids of insect cell membranes, which allows the fungus to enter the insect's hemocel and infect its tissues (Santi et al. 2010).

A key factor in the virulence of EPF is proteolytic enzymes that hydrolyze peptide bonds of insect cuticles, leading to the expsore of chitin fibrils. One of the first comprehensively described proteases is subtilisin (Pr1, serine endoprotease), which degrades both cuticle proteins and modifies the surface of the cuticle, thereby facilitating spore adhesion. This endoprotease is present in *M. anisopliae* (Shah et al. 2005), *O. sinensis* (Zhang et al. 2008), and *B. bassiana* (Donatti et al. 2008). However, other proteases such as trypsin-like acid Pr2 protease, cysteine Pr4 proteinase, and metalloprotease in *M. anisopliae*; Pr1- and Pr2-like serine proteases in *B. brongniartii*, *L. lecanii, M. rileyi*, and *Aschersonia aleyrodis*; and serine elastase in *Conidiobolus coronatus* and *B. bassiana* also participate in the pathogenesis process (Zhao et al. 2016). Some studies indicate that proteases of EPF play a dual role in the infectious process. Although their main function is hydrolysis of cuticular proteins, proteases can also participate in the inactivation of antifungal proteins produced in the epidermis of insects (Sotelo-Mundo et al. 2007).

The last group of enzymes are chitinases that are classified as follows depending on the place of their action on the chitin molecule: endochitinases (hydrolyze the β-1,4-glycosidic bond inside the chitin molecule) and exochitinases (hydrolyze *N*-acetylglucosamine oligomers formed during the action of endochitinases). The combined action of endo- and exochitinases is required for the complete degradation of insect chitin. Chitinolytic enzymes have been found in numerous EPF (Duo-Chuan 2006).

Apart from proteases, lipases, and chitinases, EPF also produce other enzymes that are not directly involved in the digestion of the cuticle but play an important role in pathogenesis. One of them is acid trehalase (ATM1) that hydrolyzes trehalose, the main disaccharide of insect hemolymph, with the release of two glucose molecules, thus providing a nutrient for EPF. Inactivation of the ATM1 gene in *M. acridum* causes a significant reduction in the virulence of this strain, which is manifested as the inability of this species to grow inside the insect's body (Jin et al. 2015).

2.2.3 Secondary metabolites: role in infection and possible applications

Entomopathogenic fungi secrete abundant low-molecular-weight organic compounds called secondary metabolites, especially in response to environmental conditions. The number of produced compounds suggests that they are necessary both for maintaining the vital functions of the hosts and for effectively infecting pathogens by damaging the nervous system or reducing insect resistance (Donzelli and Krasnoff 2016). Secondary metabolites are determined using chromatographic methods combined with mass spectrometry and molecular biology techniques. On the basis of their chemical structure, they can be divided into the following groups: cyclic depsipeptides (cyclic tetradepsipeptides and cyclic hexadepsipeptides), peptides (octadepsipeptides, dipeptides, and depsipeptides), amino acid derivatives, polyketides, peptide hybrids, and terpenoids (Donzelli and Krasnoff 2016; Wang et al. 2018b) (Online resource 1). A large number of these compounds can be distinguished due to their wide occurrence and unique properties. Taking advantage of this fact, researchers keep making efforts to extend the knowledge of the mechanisms of the secondary metabolites biosynthesis and their impact on living organisms and the environment.

Destruxins (dtxs) are cyclic hexadepsipeptides isolated mainly from *Metarhizium* sp. More than 40 types of dtxs have been reported, of which dtxs A, B, and E are the most significant in the process of pathogenesis (Arroyo-Manzanares et al. 2017). Dtxs

are well known for their insecticidal activity and phytotoxicity. They show various biological properties such as antimicrobial, antiviral, antiproliferative, cytotoxic, and immunosuppressive properties. Dtxs inhibit the action of V-ATPase proton pumps, which allows them to be used in cancer therapies or osteoporosis treatment (Liu and Tzeng 2012). However, sublines of HCT116 colon carcinoma cells are resistant to dtxs A, B, and E because of changes in membrane properties and reduction in ionophoric activity during increased activation of cholesterol synthesis (Heilos et al. 2018). Dtxs are also helpful in the treatment of Alzheimer's disease as they can inhibit and prevent the generation of β-amyloid (Aβ) (Wang et al. 2012). On the other hand, their presence in food products is a concern as they can pose a threat to human health and disturb the balance of the ecosystem (Taibon et al. 2015). Dtxs have been found in strawberries, maize (Taibon et al. 2015), and potatoes (Carpio et al. 2016).

Beauvericins produced e.g., by *B. bassiana*, *I. tenuipes or I. fumosorosea* (Wang and Xu 2012; Weng et al. 2019) and bassianolide isolated from *B. bassiana* or *Lecanicillum* sp. are other cyclic hexadepsipeptides with interesting properties. On the one hand, beauvericins contribute to the contamination of cereal crops worldwide, bioaccumulate in different tissues, and enter the food chain by polluting eggs, milk, meat, and plant products, consequently leading to apoptosis, production of reactive oxygen species (ROS), or mitochondrial damage (Olleik et al. 2019). On the other hand, they have been shown to exert strong cytotoxic effect on tumor cells, inhibit the activity of *Mycobacterium tuberculosis* or *Plasmodium falciparum*, and restrict the activity of the cholesterol acyltransferase inhibitor (Urbaniak et al. 2019; Jajić et al. 2019). Bassianolide has activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and, like beauvericins, against *M. tuberculosis*.

It inhibits several cancer cell lines and the development of the human malaria parasite *Plasmodium falciparum* (Keswani et al. 2019). Additionally, it inhibits acetylcholine-induced smooth muscle contraction (Xu et al. 2009).

Cordycepine, which belongs to octadepsipeptides, is a product of secondary metabolism of, among others, *C. militaris* and *Isaria cicadae* (formerly *Cordyceps cicadae)*. It has been used in Chinese medicine for many years because of its antioxidant, anti-inflammatory, antitumor, and neuroprotective properties (Wang et al. 2019b). As cordycepine is similar to adenosine in structure, it can inhibit processes in which adenosine is involved (Xia et al. 2017). Cordycepin reduces osteoarthritis symptoms, i.e., synovial inflammation, joint pathology, and pain behavior (Ashraf et al. 2019). It has been proved that cordycepin inhibits senescence of cells in rodents through the NRF2 (nuclear factor erythroid 2-related factor 2) and AMPK (AMP-activated protein kinase) pathways in the inflammatory process. This application gives hope for the future for obviating cells aging and radiation ulcer (Wang et al. 2019b).

Oosporein isolated mainly from *B. bassiana* or *B. brongniartii*, a highly reactive oktadepsipeptide with insecticidal and antiviral activity, is an important secondary metabolite that can also be used as an antibiotic against gram-positive bacteria (Feng et al. 2015). The insecticidal activity of oosporein is based on the stimulation of infection rather than the direct elimination of insects. Oosporein produced by *B. caledonica* has immunosuppressive properties and contributes to greater and faster mortality of insects (Mc Namara et al. 2019). Oosporein from *Cochliobolus kusanoi* exerts cytotoxic effects on Madin–Darby canine kidney (MDCK) and mouse macrophage (RAW 264.7), which is mediated by ROS through oxidative stress that increases lipid peroxidation and glutathione (GSH) level (Ramesha et al. 2015).

A research study on oosporein as a siderophore in the root endophyte *Chaetomium cupreum* revealed that the compound enhances aluminum (Al) tolerance in the plant *Miscanthus sinensis* Andersson. Furthermore, it has been proved that oosporein detoxifies Al similar to that by the phytosiderophore chlorogenic acid (Haruma et al. 2019).

M. robertsii produces polyketides that are yellow compounds named as NG-391 and NG-393 (Donzelli and Krasnoff 2016). They have structure and properties similar to those of fusarins. Like fusarin C, NG-391 exerts a strong mutagenic effect on the S9 fraction from rat liver in the Ames test (Donzelli et al. 2010). Further studies on these polyketides are required as they show antiproliferative effects on K-562 human leukemia cells, resulting in the impairment of the integrity of nucleic acid biosynthesis; on the other hand, their bioactivity may be related to binding to a protein involved in nucleoside phosphate biosynthesis

or polymerization or cell death independent of caspase (Bohnert et al. 2013).

Because the secondary metabolites of EPF are a pool of compounds with diverse activity, researchers are interested in further exploring their structures, searching for new compounds, understanding their biosynthesis pathways, and conducting in-depth studies on their properties.

3 Effect of toxic compounds on entomopathogenic fungi

The widespread use of synthetic, anthropogenic substances has increased environmental pollution through accumulation of their residues. These toxic substances cause an adverse effect on almost all living organisms to a greater or lesser extent. Pesticides are one of the basic groups of harmful substances whose presence has been detected in soil and water. These compounds enter the soil through human activities intended to improve the growth of crops. These diverse chemical compounds are usually categorized according to their mechanism of action. Among these chemicals, herbicides (substances used to control unwanted plants), fungicides and insecticides (substances used for pest control) are the most predominant ones, and both of them have harmful effects on EPF.

There are several reports on the effects of toxic compounds related to insecticides, which are often used interchangeably with EPF (such as *B. bassiana* or *M. anisopliae*) to control pests. Organophosphate (profenophos and methyl demeton) and chloroorganic insecticides (indoxacarb) showed the highest detrimental effect on *B. bassiana*. Profenophos and methyl demeton inhibited *B. bassiana* growth by 93.72% and 95.17%, respectively, after 14 days of inoculation and by 83.55% and 93.86%, respectively, after 30 days of inoculation. Indoxacarb inhibited the growth of *B. bassiana* by 100% after 14 and 30 days of inoculation (Amutha et al. 2010). The organophosphate Lorsban showed the highest toxicity to *M. anisopliae* and *I. fumosorosea*. This compound caused more than 70% mycelial growth reduction and more than 80% conidial germination reduction in both the tested fungi (Asi et al. 2010). Insecticides belonging to carbamates can also adversely affect the growth of EPF. Carbamates (Lannate, Larvin) and a pyrrole insecticide (chlorfenapyr; Pirate) caused 40–60% mycelia growth reduction and 50–70% conidial germination reduction in *M. anisopliae* and *I. fumosorosea* (Asi et al. 2010). The effect of the insecticide λ-cyhalothrin, belonging to pyrethroids, is less severe, and this compound at the recommended field dose caused 13.6% and 20.6% growth inhibition in *H. nodulosa* and *B. bassiana,* respectively (Tkaczuk et al. 2015). Among the pyrethroid insecticides, the effect of deltamethrin on *B. bassiana* was studied in detail (Forlani et al. 2014). Deltamethrin at 40 μg cm^{-2} concentration caused a significant decrease in the colony-forming unit of *B. bassiana*, but the mean inhibitory concentration (IC_{50}) was 50 μg cm^{-2}. These studies also investigated the possible mechanisms of the xenobiotic's detoxification. It was observed that exposure to the xenobiotic induced two genes of cytochrome P450 (belonging to phase I detoxification) and genes of the antioxidant system such as superoxide dismutase, catalase, and glutathione S-transferase. These findings suggest that *B. bassiana* activates defense mechanisms in response to the harmful effects of deltamethrin.

Neonicotinoids, another group of insecticides, also have toxic effects on *B. bassiana.* Acetamiprid and thiamethoxam applied at average doses recommended for field application caused a significant reduction in the vegetative growth of *B. bassiana*, while the growth of *M. anisopliae* was reduced only in the presence of acetamiprid. Interestingly, among all the tested neonicotinoids, imidacloprid (highly toxic for bees and banned in many countries) did not affect germination, vegetative growth, and conidia production of the tested EPF (Neves et al. 2001). Natural insecticides are less toxic to EPF, and one of such insecticide, Spinosad (isolated from *Saccharopolyspora spinosa*), caused only approximately 7–8% mycelial growth reduction and approximately 20% conidial germination reduction in *M. anisopliae* and *I. fumosorosea* (Asi et al. 2010).

Another natural insecticide, neem oil (derived from plants) was found to have a negative effect on EPF. It was revealed that it reduced the germination ratio, vegetative growth, and conidia production in *M. anisopliae* and *B. bassiana* (Hirose et al. 2001).

Research studies on the effects of insecticides on EPF are ongoing, and their main aim is to use lower doses of insecticides together with EPF for pest control. This task is quite important because determining a dose of an insecticide lower than that commonly applied in the field and supplementing its

action with EPF will reduce the use of toxic compounds and significantly reduce global environmental pollution. Many studies have indicated the possibility of this synergistic action, but recently, some studies have criticized this approach (Subbanna et al. 2019).

It seems that interactions between insect pathogenic fungi and insecticides are more complicated and can have both positive and negative effects on pest control. The interaction of two botanical insecticides tondexir and palizin with *B. bassiana* and *L. lecanii* was shown. These compounds at field applied doses did not cause significant inhibition of fungal germination, but significantly reduced the susceptibility of *Galleria mellonella* larvae to *B. bassiana* infection. Moreover, palizin reduced the effectiveness of *L. lecanii* against *G. mellonella* larvae (Sohrabi et al. 2019).

The appropriate use of insecticides and fungi seems to be another important aspect. Studies conducted by Meyling et al. (2018) revealed that the synergistic effect between α-cypermethrin and *B. bassiana* was observed only when the EPF was applied first (with an interval of > 48 h between applications). No synergy was observed when the insecticide was applied prior to fungal exposure within 24 h (Meyling et al. 2018).

A different approach was shown in other studies where only the effect of herbicides on EPF was tested and no attention was given to the effect of these compounds on the infectious potential of the fungi. The conducted research indicated that the pesticides quizalofop-p-ethyl and glyphosate, applied at the recommended field dose, caused growth inhibition of *H. nodulosa* by 63.4% and 52.1%, respectively, and of *B. bassiana* by 43.9% and 6.5%, respectively, during 20 days of cultivation (Tkaczuk et al. 2015). Among the tested herbicides, the effect of ametryn (an s-triazine herbicide) on EPF was studied most thoroughly. This compound not only inhibited growth and slowed down the degradation of glucose by *M. brunneum* but also disrupted its carbon and nitrogen metabolism. Ametryn also induced oxidative stress in the mycelia of *M. brunneum* (Szewczyk et al. 2018).

Another problem is the widespread use of fungicides that adversely affect all soil-borne fungi due to their mode of action. For several years, intensive research has been conducted to determine side effects of fungicides on beneficial organisms used in the Integrated Pest Management programs. Fungicides (similarly to insecticides) vary in compatibility depending on the concentration of the fungicide and the entomopathogenic fungi applied.

I. fumosorosea is sensitive to copper-based fungicides containing a higher (more than 40%) percentage of equivalent metallic copper (Avery et al. 2013). The fungicides containing lower copper doses had no apparent impact on the growth of *I. fumosorosea* and *I. farinosa* but affected the infection capacity of the fungal EPF (D'Alessandro et al. 2011; Demirci et al. 2011; Avery et al. 2013). Toxic effect of copper oxide (containing 53% and 46% metallic copper) was also confirmed for *B. bassiana* and *L. muscarium* (Kouassi et al. 2003; Ali et al. 2013).

Dithiocarbamate fungicides, harbouring metals (Zn, Mn) in their molecules also have strong inhibitory effect on EPF. Mancozeb at low concentrations (5 μg mL^{-1}) was found to inhibit *I. farinosa* conidia germination. This fungicide applied at average doses recommended for field application caused growth inhibition of *B. bassiana* and *I. farinosa* (Todorova et al. 1998; Kouassi et al. 2003). Mancozeb and propineb in low doses inhibited the sporulation of *B. bassiana* (Celar and Kos 2016). Unfortunately, mancozeb also decreased mortality of insects inoculated with EPF (Kouassi et al. 2003; Celar and Kos 2016).

Fungicide chlorothalonil (a broad-spectrum of fungicide, widely used, nitro compound) seems to have little effect on *B. bassiana* growth. However, it was shown that this fungicide severely inhibited conidial germination of *I. farinosa* and *B. bassiana* even at a low concentration of 5 μg mL^{-1} (Demirci et al. 2011; Celar and Kos 2016). In other studies chlorothalonil in field doses demonstrated fungicidal activity against *B. bassiana* and *M. anisopliae* by the inhibition or reduction of growth and sporulation (Fiedler and Sosnowska 2017). This fungicide also inhibited germination of *L. muscarium* spores (at concentration 10 ug mL^{-1}) but unexpectedly it did not suppress chitinase, Pr1, Pr2 and lipase activity suggesting its possible compatibility with the fungus (Bagga et al. 2004). Similarly to chlorothalonil, another widely used fungicide captan (dicarboximide) only slightly hindered mycelial growth but severely inhibited conidial germination *B. bassiana*, *I. fumosorosea*, *L. longisporum* (Shah et al. 2009; Dara 2017). Captan applied in field doses with *B. bassiana* severely affected the mortality in *T. molitor* larvae (Dara 2017).

Triazole fungicides have a more diverse effect on EPF and among them the most toxic is tebuconazole.

This compound suppressed the growth of *B. bassiana* and *M. anisopliae* at concentration 1000 ppm and *L. lecanii* at concentration 100 ppm. Other triazole fungicides, difenaconazole, tricyclazole and myclobutanil were also toxic to *L. lecanii* and *L. muscarium* (Demirci et al. 2011; Reddy et al. 2018). Tebuconazole was highly toxic to *I. farinosa* (at concentration 1 μg mL^{-1}) and to *P. lilacinus* (at 3 μg mL^{-1}) causing both conidial germination and mycelial growth inhibition (Demirci et al. 2011). This fungicide also hindered the entomopathogenic activity of *I. farinosa* in all three life stages of the mealybug (Demirci et al. 2011) and completely inhibited the entomopathogenic activity of the fungus *Erynia neoaphidis* on pea aphid (*Acyrthosiphon pisum*) (Latteur and Jansen 2002).

Azoxystrobin (strobilurin fungicide) was found to have a negative impact on growth and sporulation of *B. bassiana* and *M. anisopliae* and to be highly deleterious to conidial production and growth of *I. fumosorosea* (D'Alessandro et al. 2011; Fiedler and Sosnowska 2017). It exhibited moderate toxicity toward *M. majus* (growth inhibition by 40%, sporulation reduction by 60%) compared to other common fungicides used in banana agro-system (Sivakumar et al. 2019). However, the influence of azoxystrobin on EPF virulence varied. This compound did not affect the insect infection by *B. bassiana,* whereas it caused a significant inhibition of insect infection by *I. fumosorosea* (Shah et al. 2009).

Recent scientific research suggests that EPF sensitivity to fungicides can be reduced by using microsclerotia instead of spores alone. Chlorothalonil and iprodione, seem to have a limited impact on the ability of microsclerotia of *M. brunneum* to grow out and produce viable conidia, compared to their impact on germination of conidia and growth of mycelium (Wu et al. 2020). Some fungicides may need to be applied some time before or after the application of EPF to minimize any suppressive effects. However, the benefit of separating the applications in time needs to be investigated in future studies.

The effects of other anthropogenic compounds such as endocrine disrupting compounds (EDCs) on EPF are relatively rarely studied. It was found that nonylphenols are toxic to *Metarhizium*. These compounds at high concentrations (50 and 100 mg L^{-1}) caused severe changes in the pellet morphology of *M. robertsii* and reduced its metabolic activity. These disruptions were associated with changes in the hyphae structure that were manifested in terms of increased vacuolization, an increase in the size and change in the shape of mitochondria, and destruction of the plasma membrane (that leads to autolysis). Changes were also noted in the thickness and composition of the cell wall (Różalska et al. 2014). Another EDC—dibutyltin—and the by-product of its dealkylation, monobutyltin, caused nitrosative and oxidative stress and changes in the lipid profiles of *M. robertsii* (Siewiera et al. 2017a; Stolarek et al. 2019).

Industrial dyes can also adversely affect the growth of EPF. Reactive remazol red, Indanthrene blue, Yellow 3RS, and Vat Novatic Grey caused a reduction in mycelium biomass. Yellow 3RS showed the lowest adverse effect on the growth of *B. bassiana*. In the presence of this dye, the amount of biomass obtained was 5.3 g L^{-1}. Reactive remazol red and Indanthrene blue were more harmful to *B. bassiana* than Yellow 3RS. Among the tested dyes, Vat Novatic Grey was the most toxic to *B. bassiana* MTCC 4580 strain (Gola et al. 2018).

Other anthropogenic substances such as biofertilizers, which seem to be harmless to the environment, may also affect the growth of soil-inhabiting EPF. Biofertilizers are products applied on the surface of a plant or in soil and contain live microorganisms that promote plant growth and development. These products may include bacterial species such as *Rhizobium*, *Azotobacter*, and *Azospirilium* as well as blue green algae (BGA) (Kumar et al. 2017). Hirose et al. (2001) determined the effect of three biofertilizers (E.M.-4, Multibion™, and Supermagro) on two species of EPF: *M. anisopliae* and *B. bassiana*. E.M.-4 and Multibion™ reduced the germination ratio, vegetative growth, and conidia production in *M. anisopliae*. Multibion™ was found to be moderately toxic to *B. bassiana* (Hirose et al. 2001).

4 Removal of toxic pollutants by entomopathogenic fungi

Entomopathogenic fungi are commonly found in natural environment, where they come in contact with various anthropogenic pollutants. Although the mechanisms regulating the pathogenesis of insects by EPF have been relatively well understood, only a few studies have reported the interaction of toxic

impurities with these microorganisms. One of the most harmful groups of anthropogenic compounds are EDCs. They are natural or synthetic substances which, because of their structural similarity and ability to bind to hormone receptors in the cell, can disrupt the functioning of the human and animal endocrine system. This group includes, among others, alkylphenols (e.g., nonylphenol), organotin compounds (tributyl tin [TBT]), synthetic estrogens (e.g., 17α-ethinylestradiol [EE2]), and pesticides (e.g., ametryn) (Różalska et al. 2015; Szewczyk et al. 2018; Nowak et al. 2019; Stolarek et al. 2019).

4.1 Removal of 4-*n*-nonylphenol by entomopathogenic fungi

Nonylphenols are xenoestrogens widely used in the manufacture of many household products. The most commonly used nonylphenols are para-nonylphenols (4-nonylphenols [4-NP]), which occur in the form of several dozen isomers differing in the degree of branching of the alkyl fragment of the molecule. These compounds are extremely harmful for the natural environment because of their high toxicity to aquatic organisms and the ability to accumulate. They enter the environment primarily as biodegradable products of surfactants and detergents. 4-NPs also disrupt the functioning of the human and animal endocrine system by imitating 17β-estradiol and binding to estrogen receptors. These compounds have a negative effect on microorganisms and soil invertebrates and restrict plant growth (Acir and Guenther 2018).

Strains of the genus *Metarhizium* can degrade the unbranched isomer of nonylphenol (4-*n*-NP). *M. robertsii* and *M. brunneum* showed the highest activity in cultures after 24 h incubation with the compound, with more than 90% loss of substrate (Różalska et al. 2013). The extracts of *M. robertsii* culture showed 35 derivatives that were formed after the degradation of this xenobiotic (Różalska et al. 2015; Nowak et al. 2019). The proposed degradation pathway indicates that the fungi of the genus *Metarhizium* have unique capabilities to perform numerous, simultaneous mono- and dihydroxylation reactions both in the aliphatic chain and the 4-*n*-NP aromatic ring (Nowak et al. 2019). In addition, these fungi completely degraded nonylphenol in the culture environment to form CO_2 and H_2O (Różalska et al. 2015; Nowak et al. 2019).

4.2 Triazine removal by entomopathogenic fungi

Triazines are herbicides that possess a heterocyclic ring containing three unsaturated nitrogen atoms. These compounds have a carcinogenic effect and disrupt the functioning of the endocrine system in living organisms; therefore, their use is prohibited in the European Union, although their presence is still found in the natural environment. The conducted studies showed the ability of *M. brunneum* to partially decompose ametryn, a triazines, at an initial concentration of 100 mg L^{-1} to generate four derivatives (Szewczyk et al. 2018).

4.3 Dibutyltin removal by entomopathogenic fungi

Dibutyltin (DBT) is used as a curing agent for silicone rubbers and as a catalyst in esterification reactions. It enters the environment through polyvinyl chloride (PVC) materials and as a degradation product of TBT. DBT exhibits immunotoxic, neurotoxic, and pro-oxidative properties. Aerated cultures of *M. robertsii* can completely biodegrade DBT (added at an initial concentration of 20 mg L^{-1}) to monobutyltin (MBT) and hydroxylated MBT. The addition of vitamin C caused a decrease in oxidative stress associated with the presence of tin compounds in the medium, and the tested strain in the presence of this vitamin degraded MBT threefold faster (Siewiera et al. 2017a). The presence of natural estrogens—estrone (E1) and 17β-estradiol (E2)—accelerated the degradation of DBT. Moreover, both the tested estrogens protected EPF against oxidative stress caused by the presence of MBT in the culture medium (Siewiera et al. 2017b).

The cell membrane fluidity of *M. robertsii* raises the possibility of this EPF to degrade toxic substances. A membrane with a less compact structure, and thus with higher permeability, allows the transport of toxic compounds into the cell, where they are degraded (Stolarek et al. 2019).

4.4 Synthetic estrogen removal by entomopathogenic fungi

EE2 is a synthetic estrogen ubiquitously present in the environment (soil, groundwater, rivers, lakes, and seas) because of its widespread use and resistance to biodegradation. It is a derivative of E2 and is used in

hormonal contraception. Various strains of *M. robertsii* can partially remove EE2 from the growth medium with significant efficiency, which reaches up to 85% (Różalska et al. 2015).

4.5 Removal of hydrocarbons (*n*-alkanes) by entomopathogenic fungi

Entomopathogenic fungi can assimilate *n*-alkanes. The metabolic pathways of the decomposition of these compounds and the enzymes involved in this process are relatively well known (Keyhani 2018). These hydrocarbons are xenobiotics commonly found in the environment; they are also found in small amounts in the insect epicuticle, which, together with the wax layer, is the first important barrier to overcome for EPF. Lipids containing long hydrocarbon chains are present in all external insect layers. The fungus *B. bassiana* can use *n*-alkanes (extracted from insect surfaces) as a carbon source. These compounds are first hydroxylated by cytochrome P450 present in the microsomal fraction and then catabolized by β-oxidation. This fungus can also grow and metabolize synthetic straight-chain alkanes (up to *n*-C33) (Huarte-Bonnet et al. 2015). Other EPF, e.g., *M. robertsii*, are also capable of using these linear hydrocarbons (e.g., *n*-hexadecane or *n*-octacosan) as the only source of carbon and energy, and one of the first stages of degradation is carried out by cytochrome P450 (Huarte-Bonnet et al. 2018).

4.6 Removal of industrial dyes by entomopathogenic fungi

Gola et al. (2018) tested the ability of the EPF *B. bassiana* MTCC 4580 to remove four industrial dyes (Reactive remazol red, Indanthrene blue, Yellow 3RS, and Vat Novatic Grey) from wastewater. The strain *B. bassiana* MTCC 4580 could eliminate the dyes (added to the cultures at a high initial concentration of 500 mg L^{-1}) with high efficiency, which reached even 97%. Maximum removal was observed for Indanthrene blue (97%), while the removal of Vat Novatic Grey and Reactive remazol red was 91% and 84%, respectively. The mechanism of synthetic dye removal by *B. bassiana* is based on bioaccumulation and not on biodegradation (Gola et al. 2018).

5 Influence of heavy metals on entomopathogenic fungi

Entomopathogenic fungi can be used for heavy metal removal from the environment by improving the phytoremediation properties of plants (Farias et al. 2019). Even trace amounts of metals in the environment can have an adverse effect on soil, the ecosystem, water circulation, and elements in the environment as well as on human and animal health. The presence of heavy metals in the human body can contribute to the development of autoimmune diseases, digestive system disorders, and diseases of many important internal organs, including the heart, liver, kidneys, and lungs. An increase in heavy metal occurrence in the environment is due to the development of mining and electroplating industries, vehicular traffic, and waste (Hussein et al. 2011; Gola et al. 2016). The removal of heavy metal residues from the environment poses problems because of their tendency to accumulate in soil. The use of physicochemical methods of metal removal is expensive and unfavorable for the environment and uneconomic when the metal concentration is low.

The EPF *B. bassiana* has confirmed ability to remove heavy metals from contaminated water. This fungal species can remove several heavy metals such as Cu (II), Ni (II), Cd (II), Zn (II), Cr (VI), and Pb (II) added at the initial concentration of 30 mg L^{-1} with good efficiency ranging from 58% to 75%. This study also revealed the bioaccumulation of Pb (II) inside *B. bassiana* cells, although the exact mechanism of this phenomenon has not yet been proposed (Gola et al. 2018). Lead was also found to cause severe morphological alterations such as an increase in surface roughness and hyphae density and aggregation. It was also found that heavy metals are not very toxic to *B. bassiana* and the minimum inhibitory concentrations (MIC) were as follows: Zn (II): 250 mg L^{-1}; Cd (II): 200 mg L^{-1}; Cu (II), Ni (II) and Cr (VI): 100 mg L^{-1} (Gola et al. 2018).

The mechanism of *B. bassiana* susceptibility to Cr (III) has been partly explained by the studies on the role of the aldo–keto reductase Bdakr1 (Wang et al. 2018b). Like other heavy metals, chromium causes oxidative stress in fungal cells and intracellular lipid peroxidation, which leads to the accumulation of malonaldehyde (MDA). Under the normal condition, intracellular MDA levels were similar between the

wild type and ΔBbakr1. The presence of the heavy metal (5 mM Cr $(NO_3)_3$) in the culture medium caused a significant increase in MDA levels in both the wild type and ΔBbakr1 cells, but in the case of the mutant, the levels were significantly higher than those in the wild type. A growth reduction of ΔBbakr1 strain was also noted. These data suggest an impaired ability of the mutant cells to detoxify the heavy metal by catalyzing reduction reactions of aldehydes or ketones to primary and secondary alcohols (Wang et al. 2018a).

The biomass of two EPF (*B. bassiana* AUMC 3263 and *M. anisopliae* AUMC 3085) can also serve as an efficient biosorbent for Pb (II) and Cd (II) from aqueous metal solutions. The maximum lead adsorption capacities of *B. bassiana* and *M. anisopliae* were 83.33 and 66 mg g^{-1}, respectively, while for cadmium, these values reached 46 and 44 mg g^{-1}, respectively. The higher adsorption capacity of *B. bassiana* biomass than that for *M. anisopliae* could be due to the differences in the cell wall structure. The highest rate of metal absorption was observed during the first 5 min of the experiment and reached a constant value after 30 min (Hussein et al. 2011).

Heavy metals can also negatively affect the growth of EPF. Tkaczuk et al. (2019) determined the effect of five heavy metals (Zn (II), Ni (II), Cu (II), Cd (II), and Pb (II)) on the growth of *I. farinosa*, *I. fumosorosea*, and *I. tennuipes*. The experiments were conducted on Sabouraud solid medium supplemented with heavy metals at concentrations that normally occur in soil (i.e., Zn (II): 33.0; Pb(II): 13.8; Cu (II), Ni (II): 6.5; Cd (II): 0.22 mg L^{-1}) and at concentrations that were 10 and 100 times higher. The obtained data revealed that the greatest toxic effect of heavy metal ions on the EPF was observed when their concentration was 100 times higher than the natural content (on average, approximately 50.5% inhibition of growth). The greatest inhibitory effect on the growth of the fungal colonies was showed by Ni (II), while Pb (II) showed the least effects. Among all the tested species, *I. fumosorosea* had the highest tolerance to the presence of heavy metals.

Plants are widely used for bioremediation of heavy metal-contaminated areas. They interact closely with microorganisms, which can enhance plant growth and health by increasing nutrient uptake and improving plant resistance to pathogens and stress (Kidd et al. 2017). EPF are not only insect pathogens, but they are also associated with plant rhizosphere, thereby promoting plant growth. Farias et al. (2019) investigated the effect of fungal consortium containing four species of EPF (*B. bassiana, M. anisopliae, P. chlamydosporia,* and *P. lilacinum*) on the ability of *Jacaranda mimosifolia* to absorb Cu (II), Zn (II), and Mn (II). After 45 days of experiments, it was found that the use of the fungal preparation in combination with biochar caused an increase in the mass of roots and shoots of *J. mimosifolia*, intensified the translocation of metals from roots to shoots, and inhibited the leaching of metals from plant tissues, which resulted in an increased concentration of these metals in *J. mimosifolia* tissues. The application of the consortium of fungal isolates and biochar improved the phytoremediation potential of *J. mimosifolia*.

6 Nanoparticle biosynthesis by entomopathogenic fungi

During the last decade, metal nanoparticles have received considerable attention due to their wide range of applications in many different fields (e.g., pest management). They have also been used as antimicrobial agents and biomarkers. The production of nanoparticles by fungi is an interesting and ecological alternative to chemical and physical syntheses. Extracts from EPF or their culture media containing numerous enzymes can also serve as catalysts for the production of nanoparticles.

The waste mycelium after biodegradation processes is a valuable source of both enzymes and many other substances that can be reused in various processes. An example is the use of *M. robertsii* waste biomass (obtained after nonylphenol degradation) for the production of silver nanoparticles (AgNPs). Aqueous extracts from the waste mycelium *M. robertsii*, in dilutions of 25% and 50%, allowed the synthesis of silver nanoparticles from silver nitrate, and the obtained nanoparticles were small in size (from 2 to 29 nm) and relatively monodisperse, which is an important factor in their later use. The obtained colloids also exhibited antimicrobial activity, and the addition of 30 ppm AgNPs caused growth inhibition of *S. aureus* and *E. coli* (Różalska et al. 2016). These nanoparticles were also found to have fungicidal properties against *Candida albicans*, *C. glabrata*, and *C. parapsilosis* minimum inhibitory concentration

(MIC) values ranged from 1.56 to 6.25 μg mL^{-1}. An 85% decrease in viability of the tested yeast was observed after 6 h of incubation with AgNPs (added at a concentration of 6.25 μg mL^{-1}); it was also observed that this concentration of nanoparticles was not toxic to mammalian cells. The main mechanism of action of AgNPs against pathogenic yeast is probably based on changes in membrane permeability associated with a decrease in the amount of C18:2 phospholipids (Różalska et al. 2018).

In developing countries, the major public health concerns are vector-borne diseases. EPF are frequently used in protection against mosquitoes. Moreover, nanoparticles produced by these microorganisms were also found to be effective in biocontrol programs.

Myco-synthesized AgNPs are also a potential larvicidal and eco-friendly agent for controlling mosquito population. AgNPs obtained from *M. anisopliae* culture extracts may be relevant as biocontrol agents for the malaria vector *Anopheles culicifacies* mosquitos. The LC_{50} (median lethal concentration) of the studied nanoparticles ranged from 32 to 51 mg L^{-1} for various larval stages and 60 mg L^{-1} for pupae, although the low doses of silver nanoparticles limited the development of larvae and pupae (Amerasan et al. 2016). AgNPs synthesized by *I. fumosorosea* are even more effective against mosquitos. These nanoparticles were found to be more susceptible against all larval instars of *Culex quinquefasciatus* and *Aedes aegypti*. The mortality of all larval instars in the presence of 1 ppm of AgNPs ranged from 92% to 100%. The LC_{50} and LC_{90} values were 0.240–0.652 and 1.219–2.916, respectively, for all larval instars (I–IV) of *C. quinquefasciatus* and 0.065–0.137 and 0.558–1.278 ppm for *A. aegypti* (Banu and Balasubramanian 2014). The filtrates of *I. fumosorosea* can also produce iron nanoparticles that can serve as an insecticide against sweet potato whitefly *Bemisia tabaci*. These nanoparticles showed high pathogenicity against nymphs (second and third instar) and pupae, with LC_{50} values ranging from 19.17 to 37.71 ppm. LT_{50} (lethal time) at the iron nanoparticle concentration of 50 ppm was shorter for second instar nymphs (3.15 days) and longer for pupae (4.22 days) (Wang et al. 2019a).

Metarhizium anisopliae-synthesized titanium nanoparticles (TiNPs) also have insecticidal potential. The larvicidal activity of TiNPs against *G. mellonella* was 22%, but the synergistic effect of these nanoparticles and gamma-irradiated *M. anisopliae* (with higher lipase, protease, amylase, and nitrate reductase activities) caused 82% larval mortality (Yosri et al. 2018).

7 Biotransformation performed using entomopathogenic fungi

Entomopathogenic fungi can perform biotransformation, leading to the generation of derivatives with higher activity. These processes are favorable, because they improve the absorbability of products (Yang et al. 2018; Dou et al. 2019; Kozłowska et al. 2019).

7.1 Steroid biotransformation

Steroids are compounds with high economic importance, and they are widely used in medicine as hormones and active drugs. Compounds of pharmaceutical significance are obtained by modification and transformation. Because chemical methods of steroid modification are very complicated and involve even several stages, transformations are most often performed using microorganisms. Microbiological transformations of steroids include hydroxylation, Baeyer–Villiger oxidation, reduction, and isomerization. Among them, 11α-, 11β-, and 17α-hydroxylation reactions carried out by filamentous fungi are the key reactions to obtain molecules with high biological activity (Xiong et al. 2006; Gonzalez et al. 2017; Kozłowska et al. 2019).

Many filamentous fungi are used in steroid transformations, and EPF can also be efficiently used for transformations. Recent studies have shown that 12 *I. farinosa* strains could effectively transform dehydroepiandrosterone (DHEA) to 7α- and 7β-hydroxy derivatives. One of these strains, *I. farinosa* KCh KW1.1, transformed androstenediol, androstenedione, adrenosterone, 17α-methyltestosterone, 17β-hydroxyandrost-1,4,6-triene-3-one, and progesterone to mono or dihydroxy derivatives with a high yield and stereoselectivity (Kozlowska et al. 2019).

In another study, biotransformation of estrogens (E1, E2, and EE2) by *I. fumosorosea* was confirmed. Most of the estrogens were hydroxylated at C-6 position and glycosylated at C-3 position. The presence of a methyl group at the C-3 steroid ring

completely inhibited the glycosylation of estrogens by *I. fumosorosea*, indicating that a free hydroxyl group at this position is necessary for glycosylation (Kozłowska et al. 2019).

Entomopathogenic fungi can also transform steroids on a large scale, which can be used for industrial purpose in the future. 11α-Hydroxy was transformed to 13-ethyl-gon-4-ene-3,17-dione (GD, a key step in the synthesis of oral contraceptive desogestrel) by *M. anisopliae* and nano-liposome technique with a high yield. The main reaction product 11α-hydroxy-13-ethyl-gon-4-ene-3,17-dione (HGD) was also produced at high concentration of GD as 6 g L^{-1} with no reduction of transformation efficiency. The main byproduct of the reaction 6β,11α-dihydroxy derivative was found to benefit the purification of HGD (Feng et al. 2014).

Beauveria bassiana may also find applications in the pharmaceutical industry because it gives high yields of valuable 11α-hydroxy intermediates of steroids (Gonzalez et al. 2017). This process, however, seems to require optimization. In *B. bassiana* culture media with androst-4-ene-3,17-dione at pH = 6.0, only two hydroxylated metabolites were found, while in samples at pH = 7.0, four hydroxylated and/or reduced metabolites were detected. These data show that *B. bassiana* is clearly an efficient biocatalyst for 11α-hydroxylation and reduction of the 17-carbonyl group (Xiong et al. 2006). *B. bassiana* strain ATCC7159 transforms DHEA to 3β,11α,17β-trihydroxyandrost-5-ene in the presence of a moderate DHEA concentration (0.83–1.0 g L^{-1}) and in a mild environment (26 °C, pH 7) (Gonzalez et al. 2017). The addition of various compounds can positively affect the biosynthesis of these pharmaceutical metabolites. An example is the enhanced hydroxylation capacity of *B. bassiana* with *n*-alkane solvents as a carbon source. The addition of n-dodecane (n-C12) and n-hexadecane (n-C16) was found to enhance the conversion of DHEA to 3β,11α,17β-trihydroxyandrost-5-ene, resulting in the appearance 22.8% mM hydroxylated product in the culture medium. An unusual feature of *Beauveria* is the mode of conducting the reaction, which consists of the reduction of the C-17 ketone of DHEA preceded by 11α-hydroxylation. This phenomenon differs from the reaction carried out by other microorganisms, which first activate an unfunctionalized carbon to 11α-hydroxy-17-oxo derivative before obtaining a 3β,11α,17β-triol product (Gonzalez et al. 2015).

7.2 Flavonoid biotransformation

Flavonoids are derivatives of plant polyphenols. These are glycosides with O- and C-glycosidic bonds connecting the base with sugar residues (Dymarska et al. 2018a, b; Dou et al. 2019), including most often glucose, rhamnose, galactose, xylose, and arabinose (Yang et al. 2018). It is believed that the sugar group of flavonoids is the main factor affecting the absorption of these compounds by the human body (Sordon et al. 2017). There are six classes of flavonoids: flavones, isoflavones, flavanones, flavanols, flavanonols, and flavan-3-ols. Because of their antioxidant, anti-inflammatory, and antibacterial properties, they exert beneficial effects on human health. In addition, flavonoids have anti-tumor and immunomodulatory activity and protective effects on the human nervous system. The consumption of foods containing flavonoids can prevent the development of heart disease, diabetes, and depression (Dymarska et al. 2018a; Yang et al. 2018; Dou et al. 2019). They occur naturally in vegetables, fruits, cereals, wine, and tea (Yang et al. 2018; Dou et al. 2019).

Microorganisms can efficiently biotransform flavonoids to flavonoid glycosides, which are better absorbed than flavonoids themselves. Among microorganisms, EPF seem to have great potential in these processes; however, currently, *Isaria* and *Beauveria* are studied frequently, and the potential of *Metarhizium* remains undiscovered. The relevant data are presented in Table 1.

8 Conclusions

A review of literature showed that in addition to the use of EPF in biocontrol, these fungi can also be used in different ways that have not yet been described in previous reviews. EPF can also be used for removing harmful substances from the environment, because they possess enzymes that enable the removal of toxic compounds of anthropogenic origin. For example, *M. robertsii* and *M. brunneum* species can degrade nonylphenols, triazines, tin compounds, synthetic estrogen, hydrocarbons, and even industrial dyes. These microorganisms also interact with heavy

Table 1 Flavonoid transformation by entomopathogenic fungi

The group of flavonoids	Source—flavonoid	Product of transformation	Entomopathogenic fungi strain	Bibliography
Flavones	Flavone	Flavone 2-O-β-D-(4-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2	Dymarska et al. (2018a)
		4′-O-β-D-(4″-O-methyl)-glucopyranoside		
		Flavone 4′-O-β-D-(4″-O-methyl) -glucopyranoside	*I. fumosorosea* KCH J2, *I. farinosa* J1.6	
	3-hydroxyflavone	3-O-β-D-(4″-O-methyl)-glucopyranoside flavone	*I. fumosorosea* KCH J2, *I. farinosa* J1.4, *I. farinosa* J1.6	Dymarska et al. (2018b)
		3-O-β-D-glucopyranoside flavone		
		3-O-[β-D-glukopiranosyl-(1 → 6)-β-D-glucopyranosylo]-4′-hydroxyflavon	*I. fumosorosea* KCH J2	
	5-hydroxyflavone	5-hydroxyflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2, *I. farinosa* J1.4	Dymarska et al. (2018a)
	6-hydroxyflavone	6-O-β-D-(4″-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2, *I. farinosa* J1.6, *I. farinosa* KW1.2	Dymarska et al. (2018a)
	7-hydroxyflavone	7-O-β-D-(4″-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2, *I. farinosa* J1.4, *I. farinosa* J1.6	Dymarska et al. (2018a)
	5,6,7-trihydroxyflavone (Baicalein)	5,7-dihydroxyflavone 6-O-β-D-(4″-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2	Dymarska et al. (2018b)
	3-methoxyflavone	3-methoxyflavone 4′-O-β-D-(4″-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2, *I. farinosa* J1.4, *I. farinosa*	Dymarska et al. (2018b)
	7-aminoflavone	7-acetamidoflavone	*I. fumosorosea* KCH J2	Dymarska et al. (2018a)
		4′-hydroxy-7-aminoflavone	*I. fumosorosea* KCH J2	
	Luteolin	4′,5,7-trihydroxyflavone 3′-O-β-D-(4-O-methyl)-glucopyranoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
		3′,5,7-trihydroxyflavone 4′-O-β-D-(4-O-methyl)-glucopyranoside		
	Diosmetin	5,7-dihydroxy-4′-methoxyflavone 3′-O-β-D-(4-O-methyl)-glucopyranoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
	Apigenin	Mono-(4-O-methyl)-methylglucoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
Flavonols	Quercetin	3′,4′,5,7-tetrahydroxyflavone 3-O-β-D-(4″-O-methyl)-glucopyranoside	*I. fumosorosea* KCH J2	Dymarska et al. (2018b)
		3′,4′,5,7-tetrahydroxyflavone 3-O-β-D-glucopyranoside (isoquercetin)		
	Kaempferol	Mono-(4-O-methyl)-methylglucoside, monoglucoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)

Table 1 continued

The group of flavonoids	Source—flavonoid	Product of transformation	Entomopathogenic fungi strain	Bibliography
Flavanones	Naringenin	4′,5-dihydroxyflavanone 7-O-β-D-(4-O-methyl)-glucopyranoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
		5,7-dihydroxyflavanone 4′-O-β-D-(4-O-methyl)-glucopyranoside		
		5,7-dihydroxyflavanone 4′-O-β-D-glucopyranoside		
		5-hydroxyflavanone 4′,7-di-O-β-D-(4-O-methyl)-glucopyranoside		
	Hesperetin	Mono-(4-O-methyl)-methylglucoside, monoglucoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
Isoflavones	Daidzein	4″-O-methyldaidzin	*I. fumosorosea* KCH J2, *I. farinosa* J1.6, *I. farinosa* KW1.2	Dymarska et al. (2018a)
			B. bassiana AM278	Sordon et al. (2017)
	Formononetin	4-methoxyisoflavone 7-O-β-D-(4-O-methyl)-glucopyranoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
	Genistein	Mono-(4-O-methyl)-methylglucoside	*I. fumosorosea* ACCC 37814	Dou et al. (2019)
		4″-O-methylgenistin	*B. bassiana* AM278	Sordon et al. (2017)
	Biochanine A	4″-O-methylbiochanin A	*B. bassiana* AM278	Sordon et al. (2017)

metals, which sometimes adversely affect their growth or hyphae morphology; however, these fungi possess the ability to remove heavy metals from the environment, for example, from contaminated waters (zinc, copper, cadmium, nickel, and chromium) and indirectly by improving the phytoremediation properties of plants (copper, manganese, and zinc). On the basis of this current review article, the authors conclude that EPF have great potential in the biosynthesis of biologically active compounds used in medicine, for example, hydroxylated steroids and glycated flavonoids or for bioproduction of silver nanoparticles with antibacterial and antifungal properties. Because of the high potential shown by EPF and the many different functions that they can perform, they are being increasingly researched.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Acir IH, Guenther K (2018) Endocrine-disrupting metabolites of alkylphenol ethoxylates—a critical review of analytical methods, environmental occurrences, toxicity, and regulation. Sci Total Environ 635:1530–1546. https://doi.org/10.1016/j.scitotenv.2018.04.079

Ali S, Huang Z, Ren S (2013) Effect of fungicides on growth, germination and cuticle-degrading enzyme production by Lecanicillium muscarium. Biocontrol Sci Technol 23:711–723. https://doi.org/10.1080/09583157.2013.794258

Amerasan D, Nataraj T, Murugan K et al (2016) Myco-synthesis of silver nanoparticles using *Metarhizium anisopliae* against the rural malaria vector *Anopheles culicifacies* Giles (Diptera: Culicidae). J Pest Sci 89:249–256. https://doi.org/10.1007/s10340-015-0675-x

Amutha M, Gulsar Banu J, Surulivelu T, Gopalakrishnan N (2010) Effect of commonly used insecticides on the growth of white Muscardine fungus, *Beauveria bassiana* under laboratory conditions. J Biopestic 3:143–146

Araújo JPM, Hughes DP (2016) Diversity of Entomopathogenic Fungi Which groups conquered the insect body? In: Lovett B, Leger RJS (eds) Advances in genetics, vol 94. Elsevier, Amsterdam, pp 1–39. https://doi.org/10.1016/bs.adgen.2016.01.001

Arroyo-Manzanares N, Diana Di Mavungu J, Garrido-Jurado I et al (2017) Analytical strategy for determination of known and unknown destruxins using hybrid quadrupole-Orbitrap high-resolution mass spectrometry. Anal Bioanal Chem 409:3347–3357. https://doi.org/10.1007/s00216-017-0276-z

Ashraf S, Radhi M, Gowler P et al (2019) The polyadenylation inhibitor cordycepin reduces pain, inflammation and joint pathology in rodent models of osteoarthritis. Sci Rep 9:4696. https://doi.org/10.1038/s41598-019-41140-1

Asi MR, Bashir MH, Afzal M et al (2010) Compatibility of entomopathogenic fungi, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* with selective insecticides. Pak J Bot 42:4207–4214

Avery PB, Pick DA, Aristizábal LF et al (2013) Compatibility of Isaria fumosorosea (Hypocreales: Cordycipitaceae) blastospores with agricultural chemicals used for management of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). Insects 4:694–711. https://doi.org/10.3390/insects4040694

Bagga S, Hu G, Screen SE, Leger RJS (2004) Reconstructing the diversification of subtilisins in the pathogenic fungus *Metarhizium anisopliae*. Gene 324:159–169. https://doi.org/10.1016/j.gene.2003.09.031

Banu AN, Balasubramanian C (2014) Optimization and synthesis of silver nanoparticles using *Isaria fumosorosea* against human vector mosquitoes. Parasitol Res 113:3843–3851. https://doi.org/10.1007/s00436-014-4052-0

Behie SW, Bidochka MJ (2014) Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: an additional branch of the soil nitrogen cycle. Appl Environ Microbiol 80:1553–1560. https://doi.org/10.1128/AEM.03338-13

Bilgo E, Lovett B, Leger RJS et al (2018) Native entomopathogenic *Metarhizium* spp. from Burkina Faso and their virulence against the malaria vector *Anopheles coluzzii* and non-target insects. Parasites Vectors 11:11–16. https://doi.org/10.1186/s13071-018-2796-6

Bohnert M, Dahse HM, Gibson DM et al (2013) The fusarin analog NG-391 impairs nucleic acid formation in K-562 leukemia cells. Phytochem Lett 6:189–192. https://doi.org/10.1016/J.PHYTOL.2013.01.001

Carpio A, Arroyo-Manzanares N, Ríos-Moreno A et al (2016) Development of a QuEChERS-based extraction method for the determination of destruxins in potato plants by

UHPLC–MS/MS. Talanta 146:815–822. https://doi.org/10.1016/j.talanta.2015.06.008

Castro T, Mayerhofer J, Enkerli J et al (2016) Persistence of Brazilian isolates of the entomopathogenic fungi *Metarhizium anisopliae* and *M. robertsii* in strawberry crop soil after soil drench application. Agric Ecosyst Environ 233:361–369. https://doi.org/10.1016/j.agee.2016.09.031

Celar FA, Kos K (2016) Effects of selected herbicides and fungicides on growth, sporulation and conidial germination of entomopathogenic fungus *Beauveria bassiana*. Pest Manag Sci 72:2110–2117. https://doi.org/10.1002/ps.4240

D'Alessandro CP, Padin S, Urrutia MI, López Lastra CC (2011) Interaction of fungicides with the entomopathogenic fungus *Isaria fumosorosea*. Biocontrol Sci Technol 21:189–197. https://doi.org/10.1080/09583157.2010.536200

Dara SK (2017) Compatibility of the entomopathogenic fungus *Beauveria bassiana* with some fungicides used in California strawberry. Open Plant Sci J 10:29–34

Demirci F, Muştu M, Kaydan MB, Ülgentürk S (2011) Effects of some fungicides on *Isaria farinosa*, and in vitro growth and infection rate on *Planococcus citri*. Phytoparasitica 39:353–360. https://doi.org/10.1007/s12600-011-0168-2

Donatti AC, Furlaneto-Maia L, Fungaro MHP, Furlaneto MC (2008) Production and regulation of cuticle-degrading proteases from *Beauveria bassiana* in the presence of *Rhammatocerus schistocercoides* cuticle. Curr Microbiol 56:256–260. https://doi.org/10.1007/s00284-007-9071-y

Donzelli BGG, Krasnoff SB (2016) Molecular genetics of secondary chemistry in *Metarhizium* Fungi. In: Lovett B, Leger RJS (eds) Advances in genetics, vol 94. Elsevier, Amsterdam, pp 365–436

Donzelli BGG, Krasnoff SB, Churchill ACL et al (2010) Identification of a hybrid PKS–NRPS required for the biosynthesis of NG-391 in *Metarhizium robertsii*. Curr Genet 56:151–162. https://doi.org/10.1007/s00294-010-0288-0

Dou F, Wang Z, Li G, Dun B (2019) Microbial transformation of flavonoids by *Isaria fumosorosea* ACCC 37814. Molecules 24:1028. https://doi.org/10.3390/molecules24061028

Duo-Chuan L (2006) Review of fungal chitinases. Mycopathologia 161:345–360. https://doi.org/10.1007/s11046-006-0024-y

Dymarska M, Janeczko T, Kostrzewa-Susłow E (2018a) Biotransformations of flavones and an isoflavone (daidzein) in cultures of entomopathogenic filamentous fungi. Molecules 23(6):1356. https://doi.org/10.3390/molecules23061356

Dymarska M, Janeczko T, Kostrzewa-Susłow E (2018b) Glycosylation of 3-Hydroxyflavone, 3-Methoxyflavone, quercetin and baicalein in fungal cultures of the *Genus Isaria*. Molecules 23(10):pii: E2477. https://doi.org/10.3390/molecules23102477

Fan Y, Liu X, Keyhani NO et al (2017) Regulatory cascade and biological activity of *Beauveria bassiana* oosporein that limits bacterial growth after host death. Proc Natl Acad Sci USA 114:E1578–E1586. https://doi.org/10.1073/pnas.1616543114

Farias CP, Alves GS, Oliveira DC et al (2019) A consortium of fungal isolates and biochar improved the phytoremediation potential of *Jacaranda mimosifolia* D. Don and reduced copper, manganese, and zinc leaching. J Soils Sediments. https://doi.org/10.1007/s11368-019-02414-3

Feng M, Liao Z, Han L et al (2014) Enhancement of microbial hydroxylation of 13-ethyl-gon-4-ene-3,17-dione by *Metarhizium anisopliae* using nano-liposome technique. J Ind Microbiol Biotechnol 41:619–627. https://doi.org/10.1007/s10295-014-1414-7

Feng P, Shang Y, Cen K, Wang C (2015) Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc Natl Acad Sci 112:11365–11370. https://doi.org/10.1073/pnas.1503200112

Fiedler Z, Sosnowska D (2017) Side effects of fungicides and insecticides on entomopathogenic fungi in vitro. J Plant Prot Res 57:355–360. https://doi.org/10.1515/jppr-2017-0048

Forlani L, Juárez MP, Lavarías S, Pedrini N (2014) Toxicological and biochemical response of the entomopathogenic fungus *Beauveria bassiana* after exposure to deltamethrin. Pest Manag Sci 70:751–756. https://doi.org/10.1002/ps.3583

Gola D, Dey P, Bhattacharya A et al (2016) Multiple heavy metal removal using an entomopathogenic fungi *Beauveria bassiana*. Bioresour Technol 218:388–396. https://doi.org/10.1016/j.biortech.2016.06.096

Gola D, Malik A, Namburath M, Ahammad SZ (2018) Removal of industrial dyes and heavy metals by *Beauveria bassiana*: FTIR, SEM, TEM and AFM investigations with Pb(II). Environ Sci Pollut Res 25:20486–20496. https://doi.org/10.1007/s11356-017-0246-1

Gonzalez R, Nicolau F, Peeples T (2015) N-alkane Solvent-enhanced Biotransformation of Steroid DHEA by *Beauveria bassiana* as Biocatalyst. J Adv Biol Biotechnol 2:30–37. https://doi.org/10.9734/jabb/2015/13541

Gonzalez R, Nicolau F, Peeples TL (2017) Optimization of the 11α-hydroxylation of steroid DHEA by solvent-adapted *Beauveria bassiana*. Biocatal Biotransformation 35:103–109. https://doi.org/10.1080/10242422.2017.1289183

Greenfield BPJ, Lord AM, Dudley E, Butt TM (2014) Conidia of the insect pathogenic fungus, *Metarhizium anisopliae*, fail to adhere to mosquito larval cuticle. R Soc Open Sci 1(2):140193. https://doi.org/10.1098/rsos.140193

Haruma T, Yamaji K, Ogawa K et al (2019) Root-endophytic *Chaetomium cupreum* chemically enhances aluminium tolerance in *Miscanthus sinensis* via increasing the aluminium detoxicants, chlorogenic acid and oosporein. PLoS ONE 14:e0212644. https://doi.org/10.1371/journal.pone.0212644

Heilos D, Röhrl C, Pirker C et al (2018) Altered membrane rigidity via enhanced endogenous cholesterol synthesis drives cancer cell resistance to destruxins. Oncotarget 9:25661–25680. https://doi.org/10.18632/oncotarget.25432

Hirose E, Neves PMOJ, Zequi JAC et al (2001) Effect of biofertilizers and neem oil on the entomopathogenic fungi *Beauveria bassiana* (bals.) vuill. and *Metarhizium anisopliae* (Metsch.) sorok. Brazilian Arch Biol Technol 44:419–423. https://doi.org/10.1590/S1516-89132001000400013

Huarte-Bonnet C, Juárez MP, Pedrini N (2015) Oxidative stress in entomopathogenic fungi grown on insect-like

hydrocarbons. Curr Genet 61:289–297. https://doi.org/10.1007/s00294-014-0452-z

Huarte-Bonnet C, Kumar S, Saparrat MCN et al (2018) Insights into Hydrocarbon Assimilation by Eurotialean and Hypocrealean Fungi: roles for CYP52 and CYP53 Clans of Cytochrome P450 Genes. Appl Biochem Biotechnol 184:1047–1060. https://doi.org/10.1007/s12010-017-2608-z

Hussein K, Hassan S, Joo J (2011) Potential capacity of *Beauveria bassiana* and *Metarhizium anisopliae* in the biosorption of Cd^{2+} and Pb^{2+}. J Gen Appl Microbiol 57(6):347–355

Jaber LR, Enkerli J (2017) Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Sci Technol 27:28–41. https://doi.org/10.1080/09583157.2016.1243227

Jaihan P, Sangdee K, Sangdee A (2016) Selection of entomopathogenic fungus for biological control of chili anthracnose disease caused by *Colletotrichum* spp. Eur J Plant Pathol 146:551–564. https://doi.org/10.1007/s10658-016-0941-7

Jajić I, Dudaš T, Krstović S et al (2019) Emerging fusarium mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin in serbian maize. Toxins (Basel) 11:357. https://doi.org/10.3390/toxins11060357

Jin K, Peng G, Liu Y, Xia Y (2015) The acid trehalase, ATM1, contributes to the in vivo growth and virulence of the entomopathogenic fungus, *Metarhizium acridum*. Fungal Genet Biol 77:61–67. https://doi.org/10.1016/j.fgb.2015.03.013

Keswani C, Singh HB, Hermosa R et al (2019) Antimicrobial secondary metabolites from agriculturally important fungi as next biocontrol agents. Appl Microbiol Biotechnol 103:9287–9303

Keyhani NO (2018) Lipid biology in fungal stress and virulence: entomopathogenic fungi. Fungal Biol 122:420–429. https://doi.org/10.1016/j.funbio.2017.07.003

Khan S, Guo L, Maimaiti Y et al (2012) Entomopathogenic fungi as microbial biocontrol agent. Mol Plant Breed. https://doi.org/10.5376/mpb.2012.03.0007

Kidd PS, Alvarez-Lopez V, Becerra-Castro C et al (2017) Chapter three—potential role of plant-associated bacteria in plant metal uptake and implications in phytotechnologies. Adv Bot Res 83:87–126

Klieber J, Reineke A (2016) The entomopathogen *Beauveria bassiana* has epiphytic and endophytic activity against the tomato leaf miner Tuta absoluta. J Appl Entomol 140:580–589. https://doi.org/10.1111/jen.12287

Kouassi M, Coderre D, Todorova SI (2003) Effects of the timing of applications on the incompatibility of three fungicides and one isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina). J Appl Entomol 127:421–426

Kozłowska E, Dymarska M, Kostrzewa-Susłow E, Janeczko T (2019) Cascade biotransformation of estrogens by Isaria fumosorosea KCh J2. Sci Rep 9:1–8. https://doi.org/10.1038/s41598-019-47225-1

Kumar R, Kumawat N, Sahu YK (2017) Role of biofertilizers in agriculture. Pop Kheti 5:63–66

Lacey LA, Mercadier G (1998) The effect of selected allelochemicals on germination of conidia and blastospores and mycelial growth of the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes). Mycopathologia 142:17–25. https://doi.org/10.1023/A:1006963016316

Lacey LA, Grzywacz D, Shapiro-Ilan DI et al (2015) Insect pathogens as biological control agents: back to the future. J Invertebr Pathol 132:1–41. https://doi.org/10.1016/j.jip.2015.07.009

Latteur G, Jansen JP (2002) Effects of 20 fungicides on the infectivity of conidia of the aphid entomopathogenic fungus Erynia neoaphidis. Biocontrol 47:435–444. https://doi.org/10.1023/A:1015639017666

Liu BL, Tzeng YM (2012) Development and applications of destruxins: a review. Biotechnol Adv 30:1242–1254. https://doi.org/10.1016/j.biotechadv.2011.10.006

Lovett B, Leger RJS (2017) The insect pathogens. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.funk-0001-2016

Mascarin GM, Jaronski ST (2016) The production and uses of *Beauveria bassiana* as a microbial insecticide. World J Microbiol Biotechnol 32:1–26. https://doi.org/10.1007/s11274-016-2131-3

Mc Namara L, Dolan SK, Walsh JMD et al (2019) Oosporein, an abundant metabolite in *Beauveria caledonica*, with a feedback induction mechanism and a role in insect virulence. Fungal Biol 123:601–610. https://doi.org/10.1016/j.funbio.2019.01.004

McKinnon AC, Saari S, Moran-Diez ME et al (2017) *Beauveria bassiana* as an endophyte: a critical review on associated methodology and biocontrol potential. Biocontrol 62:1–17. https://doi.org/10.1007/s10526-016-9769-5

Meyling NV, Arthur S, Pedersen KE et al (2018) Implications of sequence and timing of exposure for synergy between the pyrethroid insecticide alpha-cypermethrin and the entomopathogenic fungus *Beauveria bassiana*. Pest Manag Sci 74:2488–2495. https://doi.org/10.1002/ps.4926

Mondal S, Baksi S, Koris A, Vatai G (2016) Journey of enzymes in entomopathogenic fungi. Pacific Sci Rev A Nat Sci Eng 18:85–99. https://doi.org/10.1016/j.psra.2016.10.001

Moonjely SS, Barelli L, Bidochka MJ (2016) Insect pathogenic fungi as endophytes. In: Lovett B, Leger RJS (eds) Advances in genetics. Elsevier, Amsterdam, pp 107–135

Neves PMOJ, Hirose E, Tchujo PT, Moino A (2001) Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotrop Entomol 30:263–268. https://doi.org/10.1590/s1519-566x2001000200009

Nowak M, Soboń A, Litwin A, Różalska S (2019) 4-n-nonylphenol degradation by the genus *Metarhizium* with cytochrome P450 involvement. Chemosphere 220:324–334. https://doi.org/10.1016/j.chemosphere.2018.12.114

Olleik H, Nicoletti C, Lafond M et al (2019) Comparative structure–activity analysis of the antimicrobial activity, cytotoxicity, and mechanism of action of the fungal cyclohexadepsipeptides enniatins and beauvericin. Toxins (Basel) 11:514. https://doi.org/10.3390/toxins11090514

Pedrini N, Rosana C, Patricia Juarez M (2007) Biochemistry of insect epicuticule degradation by entomopathogenic fungi. Comp Biochem Physiol 146:124–137

Ramakuwela T, Hatting J, Bock C et al (2020) Establishment of *Beauveria bassiana* as a fungal endophyte in pecan (Carya

illinoinensis) seedlings and its virulence against pecan insect pests. Biol Control 140:104102. https://doi.org/10.1016/j.biocontrol.2019.104102

Ramesha A, Venkataramana M, Nirmaladevi D et al (2015) Cytotoxic effects of oosporein isolated from endophytic fungus *Cochliobolus kusanoi*. Front Microbiol 6:870. https://doi.org/10.3389/fmicb.2015.00870

Reddy DS, Reddy M, Pushpalatha M (2018) Interaction of fungicides with bio-control agents. J Entomol Zool Stud. 3:2098–2104

Ríos-Moreno A, Garrido-Jurado I, Resquín-Romero G et al (2016) Destruxin A production by *Metarhizium brunneum* strains during transient endophytic colonisation of Solanum tuberosum. Biocontrol Sci Technol 26:1574–1585. https://doi.org/10.1080/09583157.2016.1223274

Różalska S, Pawłowska J, Wrzosek M et al (2013) Utilization of 4-n-nonylphenol by *Metarhizium* sp. isolates. Acta Biochim Pol 60:677–682

Różalska S, Glińska S, Długoński J (2014) *Metarhizium robertsii* morphological flexibility during nonylphenol removal. Int Biodeterior Biodegrad 95:285–293. https://doi.org/10.1016/j.ibiod.2014.08.002

Różalska S, Bernat P, Michnicki P, Długoński J (2015) Fungal transformation of 17α-ethinylestradiol in the presence ofvarious concentrations of sodium chloride. Int Biodeterior Biodegrad 103:77–84. https://doi.org/10.1016/j.ibiod.2015.04.016

Różalska S, Soliwoda K, Długoński J (2016) Synthesis of silver nanoparticles from *Metarhizium robertsii* waste biomass extract after nonylphenol degradation, and their antimicrobial and catalytic potential. RSC Adv 6:21475–21485. https://doi.org/10.1039/c5ra24335a

Różalska B, Sadowska B, Budzyńska A et al (2018) Biogenic nanosilver synthesized in *Metarhizium robertsii* waste mycelium extract—as a modulator of *Candida albicans* morphogenesis, membrane lipidome and biofilm. PLoS ONE 13:1–21. https://doi.org/10.1371/journal.pone.0194254

Santi L, Beys da Silva WO, Berger M et al (2010) Conidial surface proteins of *Metarhizium anisopliae*: source of activities related with toxic effects, host penetration and pathogenesis. Toxicon 55:874–880. https://doi.org/10.1016/j.toxicon.2009.12.012

Shah FA, Wang CS, Butt TM (2005) Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiol Lett 251:259–266. https://doi.org/10.1016/j.femsle.2005.08.010

Shah FA, Ansari MA, Watkins J et al (2009) Influence of commercial fungicides on the germination, growth and virulence of four species of entomopathogenic fungi. Biocontrol Sci Technol 19:743–753. https://doi.org/10.1080/09583150903100807

Siewiera P, Różalska S, Bernat P (2017a) Efficient dibutyltin (DBT) elimination by the microscopic fungus *Metarhizium robertsii* under conditions of intensive aeration and ascorbic acid supplementation. Environ Sci Pollut Res 24:12118–12127. https://doi.org/10.1007/s11356-017-8764-4

Siewiera P, Różalska S, Bernat P (2017b) Estrogen-mediated protection of the organotin-degrading strain *Metarhizium robertsii* against oxidative stress promoted by monobutyltin. Chemosphere 185:96–104. https://doi.org/10.1016/j.chemosphere.2017.06.130

Silva WOB, Santi L, Berger M et al (2009) Characterization of a spore surface lipase from the biocontrol agent *Metarhizium anisopliae*. Process Biochem 44:829–834. https://doi.org/10.1016/j.procbio.2009.03.019

Sivakumar T, Jiji T, Naseema A (2019) Effect of pesticides used in banana agro-system on entomopathogenic fungus, *Metarhizium majus* Bisch, Rehner and Humber. Int J Trop Insect Sci. https://doi.org/10.1007/s42690-019-00080-z

Skinner M, Parker BL, Kim JS (2014) Role of entomopathogenic fungi. In: Abrol DP (ed) Integrated pest management. Academic Press, Cambridge, pp 169–191

Sohrabi F, Jamali F, Morammazi S et al (2019) Evaluation of the compatibility of entomopathogenic fungi and two botanical insecticides tondexir and palizin for controlling *Galleria mellonella* L. (Lepidoptera: Pyralidae). Crop Prot 117:20–25

Sordon S, Popłónski J, Tronina T, Huszcza E (2017) Microbial glycosylation of daidzein, genistein and biochanin a: two new glucosides of biochanin A. Molecules 22(1):pii: E81. https://doi.org/10.3390/molecules22010081

Sotelo-Mundo RR, Lopez-Zavala AA, Garcia-Orozco KD et al (2007) The lysozyme from insect (Manduca sexta) is a cold-adapted enzyme. Protein Pept Lett 14:774–778

Stolarek P, Różalska S, Bernat P (2019) Lipidomic adaptations of the *Metarhizium robertsii* strain in response to the presence of butyltin compounds. Biochim Biophys Acta—Biomembr 1861:316–326. https://doi.org/10.1016/j.bbamem.2018.06.007

Subbanna ARNS, Stanley J, Venkateswarlu V et al (2019) Toxicological prospects on joint action of microbial insecticides and chemical pesticides. In: Khan MA, Ahmad W (eds) Microbes for sustainable insect pest management. Springer, Cham, pp 317–340. https://doi.org/10.1007/978-3-030-23045-6_12

Supakdamrongkul P, Bhumiratana A, Chanpen W (2010) Characterization of an extracellular lipase from the biocontrol fungus, *Nomuraea rileyi* MJ, and its toxicity toward *Spodoptera litura*. J Invertebr Pathol 105:228–235

Szewczyk R, Kuśmierska A, Bernat P (2018) Ametryn removal by Metarhizium brunneum: biodegradation pathway proposal and metabolic background revealed. Chemosphere 190:174–183. https://doi.org/10.1016/j.chemosphere.2017.10.011

Taibon J, Sturm S, Seger C et al (2015) Quantitative assessment of destruxins from strawberry and maize in the lower parts per billion range: combination of a QuEChERS-based extraction protocol with a fast and selective UHPLC-QTOF-MS assay. J Agric Food Chem 63:5707–5713. https://doi.org/10.1021/acs.jafc.5b01562

Tigano-Milani MS, Carneiro RG, De Faria MR et al (1995) Isozyme Characterization and Pathogenicity of *Paecilomyces fumosoroseus* and P. lilacinus to Diabrotica speciosa (Cleoptera: Chrysomelidae) and Meloidogyne javanica (Nematoda: Tylenchidae). Biol Control 5:378–382

Tkaczuk C, Harasimiuk M, Król A, Beres PK (2015) The effect of selected pesticides on the growth of entomopathogenic

fungi *Hirsutella nodulosa* and *Beauveria bassiana*. J Ecol Eng 16:177–183. https://doi.org/10.12911/22998993/2952

Tkaczuk C, Majchrowska-Safaryan A, Panasiuk T, Tipping C (2019) Effect of selected heavy metal ions on the growth of entomopathogenic fungi from the *Genus Isaria*. Appl Ecol Environ Res 17:2571–2582. https://doi.org/10.15666/aeer/1702_25712582

Todorova SI, Coderre D, Duchesne RM, Côté JC (1998) Compatibility of *Beauveria bassiana* with selected fungicides and herbicides. Environ Entomol 27:427–433. https://doi.org/10.1093/ee/27.2.427

Urbaniak M, Stępień Ł, Uhlig S (2019) Evidence for naturally produced beauvericins containing *N*-methyl-tyrosine in *Hypocreales* fungi. Toxins (Basel) 11:182. https://doi.org/10.3390/toxins11030182

Vega FE (2018) The use of fungal entomopathogens as endophytes in biological control: a review. Mycologia 110:4–30. https://doi.org/10.1080/00275514.2017.1418578

Wang C, Leger RJS (2007) The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. Eukaryot Cell 6:808–816. https://doi.org/10.1128/EC.00409-06

Wang Q, Xu L (2012) Beauvericin, a bioactive compound produced by fungi: a short review. Molecules 17:2367–2377. https://doi.org/10.3390/molecules17032367

Wang B, Kang Q, Lu Y et al (2012) Unveiling the biosynthetic puzzle of destruxins in Metarhizium species. Proc Natl Acad Sci 109:1287–1292. https://doi.org/10.1073/pnas.1115983109

Wang H, He Z, Luo L et al (2018a) An aldo-keto reductase, Bbakr1, is involved in stress response and detoxification of heavy metal chromium but not required for virulence in the insect fungal pathogen, *Beauveria bassiana*. Fungal Genet Biol 111:7–15. https://doi.org/10.1016/j.fgb.2018.01.001

Wang X, Gong X, Li P et al (2018b) Structural diversity and biological activities of cyclic depsipeptides from fungi. Molecules 23:169. https://doi.org/10.3390/molecules23010169

Wang X, Xu J, Wang X et al (2019a) *Isaria fumosorosea*-based zero-valent iron nanoparticles affect the growth and survival of sweet potato whitefly, *Bemisia tabaci* (Gennadius). Pest Manag Sci 75:2174–2181

Wang Z, Chen Z, Jiang Z et al (2019b) Cordycepin prevents radiation ulcer by inhibiting cell senescence via NRF2 and AMPK in rodents. Nat Commun 10:2538. https://doi.org/10.1038/s41467-019-10386-8

Weng Q, Zhang X, Chen W, Hu Q (2019) Secondary metabolites and the risks of *Isaria fumosorosea* and *Isaria farinosa*. Molecules 24(4):pii: E664. https://doi.org/10.3390/molecules24040664

Wu S, Kostromytska OS, Goble T et al (2020) Compatibility of a microsclerotial granular formulation of the entomopathogenic fungus *Metarhizium brunneum* with fungicides. Biocontrol 9:113–123. https://doi.org/10.1007/s10526-019-09983-9

Xia Y, Luo F, Shang Y et al (2017) Fungal cordycepin biosynthesis is coupled with the production of the safeguard molecule pentostatin. Cell Chem Biol 24:1479–1489.e4. https://doi.org/10.1016/j.chembiol.2017.09.001

Xiong Z, Wei Q, Chen H et al (2006) Microbial transformation of androst-4-ene-3,17-dione by *Beauveria bassiana*. Steroids 71:979–983. https://doi.org/10.1016/j.steroids.2006.07.007

Xu Y, Orozco R, Wijeratne EMK et al (2009) Biosynthesis of the cyclooligomer depsipeptide bassianolide, an insecticidal virulence factor of *Beauveria bassiana*. Fungal Genet Biol 46:353–364. https://doi.org/10.1016/j.fgb.2009.03.001

Yang B, Liu H, Yang J et al (2018) New insights on bioactivities and biosynthesis of flavonoid glycosides. Trends Food Sci Technol 79:116–124. https://doi.org/10.1016/j.tifs.2018.07.006

Yosri M, Abdel-Aziz MM, Sayed RM (2018) Larvicidal potential of irradiated myco-insecticide from *Metarhizium anisopliae* and larvicidal synergistic effect with its mycosynthesized titanium nanoparticles (TiNPs). J Radiat Res Appl Sci 11:328–334. https://doi.org/10.1016/j.jrras.2018.06.001

Zhang YJ, Liu X, Wang M (2008) Clonig, expression and characterization of two novel cuticle-degrading serine proteases from the entomopathogenic fungus Cordyceps sinensis. Res Microbiol 159:462–469

Zhao H, Lovett B, Fang W (2016) Genetically engineering entomopathogenic fungi. In: Lovett B, Leger RJT (eds) Advances in genetics, vol 94. Elsevier, Amsterdam, pp 137–163

Zimmermann G (2008) The entomopathogenic fungi Isaria farinosa (formerly Paecilomyces farinosus) and the Isaria fumosorosea species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Sci Technol 18:865–901. https://doi.org/10.1080/09583150802471812

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Lipidomic response of the entomopathogenic fungus *Beauveria bassiana* to pyrethroids

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Pyrethroids are chemical insecticides that are widely used to control pests. Entomopathogenic fungi are considered environmentally safe alternatives to these compounds. Pyrethroids and entomopathogenic fungi not only co-exist in the environment but can also be applied together in pest control. They are often found in contact with each other, and thus, it seems important to understand their interactions at the cellular level. In this study, we analyzed whether pyrethroids could influence the phospholipid profile of *Beauveria bassiana* and whether membrane changes are one of the mechanisms by which these fungi adapt to unfavorable environmental conditions. The results of our study revealed that pyrethroids changed the phospholipid profile and increased the cell membrane permeability of *B. bassiana*, which enabled them to enter and accumulate within the fungal cells, resulting in oxidative stress. Pyrethroids influenced the amount of neutral lipids, caused a decrease in sodium content, and also temporarily lowered the level of the secondary metabolite oosporein in the studied fungi. These findings indicate that the effect of pyrethroids on entomopathogenic fungi may be more complex than originally thought and that lipidomic studies can aid in fully understanding the influence of these chemicals on the mentioned group of fungi.

Pyrethroids are insecticides used to control arthropods in agriculture, forestry, and greenhouses. They are neurotoxic substances that disrupt the nervous system of insects by affecting the activity of sodium and chloride channels[1–3]. Due to the widespread use as well as the lipophilic and hydrophobic properties of pyrethroids, their residues can be found in the environment (e.g., soil, surface water, and sediments)[2,4]. Despite the fact that pyrethroids are less harmful and safer than other insecticides, it has been proven that they exert negative effects on living organisms[4]. Pyrethroids have rather long half-lives in soil and are also poorly soluble in water due to their strongly hydrophobic nature[2].

The soil is the habitat of many microorganisms, including entomopathogenic fungi, which are parasites controlling the density of insect populations in nature. It has been estimated that the propagules of *Ascomycota* entomopathogenic fungi could reach 10^6 per g of soil[5]. Entomopathogens of the genera *Beauveria* and *Metarhizium* are widely used as bioinsecticides and introduced into the environment to protect crops[6]. Due to their long-term action, these fungi can be used together with chemical insecticides or as their alternatives in pest control[7].

Entomopathogenic fungi and pyrethroids can not only be applied together to control pests but also coexist in the environment, and are therefore often found in contact with each other. Thus, it seems important to understand their interactions at the cellular level.

So far, only a few studies have analyzed the response of entomopathogenic fungi to pyrethroids at the cellular level, with most of them focusing only on fungal growth in the presence of these compounds[8–11]. Pyrethroids do not mostly adversely affect fungal growth[12], and hence are considered to not cause any significant cell damage. Forlani et al.[8] revealed that exposure of *Beauveria bassiana* to the pyrethroid deltamethrin led to the induction of two cytochrome P450 genes and antioxidant system genes such as superoxide dismutase, catalase, and glutathione S-transferase. These findings suggest that the defense mechanisms of *B. bassiana* are activated in response to the harmful effects of deltamethrin.

In this study, we analyzed whether pyrethroids, which are considered nontoxic to entomopathogenic fungi, influenced the phospholipid profile of *B. bassiana* due to the fact that changes in the membrane structure and properties are one of the mechanisms that allow microorganisms to adapt to unfavorable environmental

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conditions and that even slight changes in lipidome may affect the functioning of *B. bassiana* fungi in the natural environment.

Materials and methods

Reagents. The insecticides used in the study were λ-cyhalothrin, α-cypermethrin, and deltamethrin. Stock solutions of pyrethroids were prepared in 10 mg mL^{-1} acetonitrile. The other reagents used were phosphate-buffered saline (PBS), propidium iodide, ammonium formate, formic acid, cholesterol, BSTFA/TMCS solution (N,O-bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane), DAB (3,3′-diaminobenzidine tetrahydrochloride hydrate), NBT (Nitrotetrazolium Blue chloride), sodium dihydrogen phosphate and disodium hydrogen phosphate dodecahydrate, chloroform, methanol, methyl tert-butyl ether, isopropyl alcohol, methanolic KOH, HCl, and nitric acid.

Strain and growth conditions. The strain *Beauveria bassiana* ARSEF 2860 used in the study was obtained from the ARSEF (Agricultural Research Service Collection of Entomopathogenic Fungal Cultures, USA). It was isolated from *Schizaphis graminum* and can be potentially applied in the biological control of aphids and spider mites[13,14]. Moreover, its genome has been sequenced and is therefore commonly used in scientific research[15–17]. For the experiments, conidia were harvested from 14-day-old cultures of *B. bassiana* grown on Sabouraud dextrose agar slant and transferred to 40 mL Sabouraud dextrose broth medium at a density of 10^6 per mL. The medium was supplemented with the abovementioned insecticides at final concentrations of 5, 50, and 100 mg L^{-1}. The inoculated medium was incubated in a rotary shaker (120 rpm) for 24, 36, and 48 h at 28 °C.

Dry mass measurements, metabolic activity analysis, and microscopy. The effect of pyrethroids on the growth of *B. bassiana* was determined by measuring the dry mass of cultures. After incubation for 36 and 48 h, the cultures were filtered under reduced pressure through a Whatman membrane filter (0.45 µm) and dried at 100 °C for 1 h to achieve a constant weight. The effect of pyrethroids on the viability (metabolic activity) of *B. bassiana* was determined by a method using fluorescein diacetate (FDA) with additional modifications[18]. Briefly, the cultures were filtered using a vacuum pump, and the entire wet mass from the flask was divided into portions and transferred to the wells of a 24-well plate. To each well, 1 mL of FDA in sodium phosphate buffer (pH 6.4, 0.7 mg mL^{-1}) was added. The samples were incubated in the dark at 28 °C for 15 min, and the fluorescence of the wells was then read using a plate reader at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The fluorescence values were divided by the amount of dry mass estimated for each sample. The results were expressed as % viability in comparison to the biotic control.

Microscopic observations were made on samples mounted on slides. Images were captured in a Nikon Eclipse E200 microscope equipped with a digital camera and Nikon E Plan objective (40x/0.6) with DeltaPix InSight software.

Determination of sodium, magnesium, potassium, and calcium. The effect of pyrethroids on the content of sodium, magnesium, potassium, and calcium of entomopathogenic fungi was determined in mycelium originating from the insecticide-exposed cultures. Briefly, mycelia were separated from cultures using a filter paper and dried at 100 °C to obtain dry weight. Subsequently, the biomass was mineralized with concentrated nitric acid (65%). The content of metals in the samples was estimated by atomic absorption spectrometry (AAS) using a Spectra 240 FS apparatus.

Ergosterol measurement. To determine the content of ergosterol in fungal biomass, 100 mg of biomass was placed in an Eppendorf tube with glass beads. The samples were frozen in liquid nitrogen and then homogenized using a FastPrep-24 instrument for 20 s at 5 m s^{-1}. Ergosterol was extracted as described by Stolarek et al.[19] with additional modifications. Briefly, 1 mL of methyl tert-butyl ether–methanol mixture (3:1, v/v) was added to the samples and the samples were vortexed. Next, 650 µL of water–methanol mixture (3:1, v/v) was added, and the samples were vortexed again and centrifuged at 5000 × *g* for 5 min at 10 °C. After centrifugation, the top layer was collected and evaporated. Then, the samples were dissolved in a mixture of 0.5 mL of chloroform, 0.5 mL of methanolic KOH, and 20 µg of cholesterol (20 µL from stock solution at the concentration of 1 mg mL^{-1}). After incubating the samples for 1 h at 23 °C, 0.325 mL of 1 M HCl and 0.125 mL of deionized water were added and centrifuged (5000 × g). Subsequently, the lower layer was transferred to a new Eppendorf tube and dried for 12 h under fume hood. Then, 100 µL of the BSTFA/TMCS solution was added and the samples were incubated for 90 min at 85 °C. After incubation, 50 µL of hexane was added and the samples were transferred to chromatographic tubes. The content of ergosterol in the samples was determined by gas chromatography–tandem mass spectrometry as described previously using an Agilent 7890 system equipped with an HP 5 MS column and a 5975C mass detector[20].

Phospholipid analysis. For analyzing the phospholipid profile, samples were extracted as described in the previous section. After evaporation, the extract was dissolved in 1 mL of methanol and analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using an LC Agilent 1200 system coupled with a Sciex QTRAP 4500 tandem mass spectrometer. A Kinetex C18 column (50 mm × 2.1 mm, particle size 5 µm) heated to 40 °C with a flow rate of 500 µL min^{-1} was used for this purpose. The ion source of the mass spectrometer was operated in a negative mode under the following conditions: spray voltage 4.500 V, curtain gas 25, nebulizer gas 60, auxiliary gas 50, and temperature 600 °C.

Membrane permeability assay. For determining membrane permeability, 1 mL of each culture was transferred to an Eppendorf tube and the samples were centrifuged. The supernatant was removed, and 1 mL of PBS and 2 µL of propidium iodide at a concentration of 0.1 mg mL^{-1} were added. Subsequently, the samples were incubated in the dark at room temperature for 5 min. After incubation, the mycelium was washed twice in PBS, suspended in 1 mL of PBS, and transferred to a 24-well titration plate. Fluorescence of the samples was measured using a FLUOstar Omega fluorescence microplate reader (excitation wavelength: 540 nm, emission wavelength: 610 nm), with the fluorescence of the supernatant set as a background. The results were expressed as a fluorescence unit (U) per mg of dry mass.

Extraction and quantification of pyrethroids. For estimating the content of pyrethroids, the medium was separated from the biomass by filtration and extracted with ethyl acetate followed by methylene chloride. The amount of insecticides in the mycelium and culture medium was determined using a gas chromatography–mass spectrometry system equipped with an HP 5 MS column (30 m × 250 µm × 0.25 µm) and a 5975C mass detector.

Quantification of neutral lipids. Triacylglicerols (TAGs) and diacylglycerols (DAGs) were extracted as described in "Ergosterol measurement" section. After evaporation, the samples were dissolved in 1 mL of methanol. The content of acylglycerols was determined by LC–MS/MS. To detect acylglycerol, ammonium adducts of multiple reaction monitoring (MRM) scans including parent–daughter pairs were used. Chromatographic separation was conducted on a C18 column heated to 40 °C, and detection was performed by single-ion monitoring and the enhanced product ion method. Water and a mixture of acetonitrile–isopropyl alcohol (5:2) containing 5 mM ammonium formate and 0.1% formic acid were used as mobile phases[19].

Oxidative stress. To determine the content of hydrogen peroxide, 1 mL of the culture was centrifuged at 2000 × *g* for 5 min. The obtained biomass was suspended in 1 mL of DAB solution (1 mg mL^{-1} of DAB was dissolved in sodium phosphate buffer (pH 7.8) and pH was adjusted to 3.8 before adding the solution to the biomass, and the samples were incubated in sunlight for 1 h.

The content of superoxide anion in *B. bassiana* hyphae was measured using the method described by Nykiel-Szymańska et al.[21] Briefly, 1 mL of the culture was centrifuged at 2000 × *g* for 5 min. The obtained biomass was suspended in 1 mL of the solution containing 0.1% NBT and 10 mM sodium azide dissolved in sodium phosphate buffer (pH 7.8). The samples were incubated for 1 h in the dark at room temperature.

The content of hydrogen peroxide and superoxide anion in *B. bassiana* hyphae was examined under an LSM510 Meta confocal laser scanning microscope. The results were expressed as a percentage of the stained area compared to the total hyphal area.

Oosporein determination. To determine the content of oosporein pigment, the cultures were centrifuged at 5000 × *g* for 15 min. Then, the medium was acidified to pH 2 with hydrochloric acid. The acidified medium was extracted twice with ethyl acetate for 3 min, and the obtained extracts were dehydrated with anhydrous sodium sulfate. Next, the extracts were filtered through a paper filter, and the filtrate was evaporated using a vacuum evaporator. Subsequently, the samples were dissolved in 1 mL of methanol and analyzed using an LC Agilent 1200 system coupled with a Sciex QTRAP 3200 tandem mass spectrometer.

Flow injection analysis of samples was conducted with an injection volume of 10 µL and a flow rate of 600 µL min^{-1}. A mixture of water with 0.1% formic acid (solvent A) and a mixture of acetonitrile with 0.1% formic acid (solvent B) were used as mobile phases. A phase A–phase B ratio of 30:70 was maintained for 0.7 min. Tandem mass spectrometry detection was conducted using the electrospray ion source ESI with negative polarization. To confirm the presence of oosporein, the three following MRM pairs were determined: Q1/Q3—305.1/177.1, 305.1/149.1, and 305.1/133.1. Of these, the quantitative pair was 305.1/177.1 The individual analytes parameters were: entrance potential (EP; (-5)), collision cell entrance potential (CEP; (-30)), collision energy (CE; (-30)), collision cell exit potential (CXP; (-1)), declustering potential (DP (-35)). The amount of oosporein was determined based on a calibration curve over a concentration range of 1–100 µg, with quadratic regression, where the "*R*" value was 0.9954.

Statistical analyses. All samples were prepared in triplicate. The experiments were repeated twice, and the standard deviation (SD) of the results was determined. The normality of the data distribution was determined by the Shapiro–Wilk test. Data were analyzed using one-way analysis of variance (ANOVA), and the means were compared using Tukey's post hoc test. A p-value of < 0.05 was considered statistically significant. Statistical analyses were conducted in the STATISTICA version 13.3 software (StatSoft).

Results and discussion

Effect of pyrethroids on *B. bassiana* dry biomass content and metabolic activity. After 36 h of incubation, statistically significant ($P < 0.01$) changes in biomass dry weight were observed in the *B. bassiana* samples supplemented with pyrethroids at the concentrations of 50 and 100 mg L^{-1} (Supplementary Table S1). However, after 48 h of incubation, statistically significant changes were noted only in the samples supplemented with 100 mg $L^{-1}$of λ-cyhalothrin ($P = 0.00169$) and α-cypermethrin ($P = 0.015635$). The results of our study also indicated that the tested pyrethroids did not affect the growth of *B. bassiana* at the concentration of 5 mg L^{-1}.

Intriguingly, microscopic observations did not reveal any morphological changes in mycelia exposed to pyrethroids (Supplementary Fig S1 and Supplementary Fig S2) but we noted a significant decrease in the number

	Control	Pyrethroids (100 mg L^{-1})		
		λ-cyhalothrin	α-cypermethrin	Deltamethrin
24 h	100.00 ± 4.76	135.73 ± 6.58**	132.46 ± 19.00**	146.85 ± 12.33**
36 h	100.00 ± 3.85	111.11 ± 11.76*	88.76 ± 12.80	98.26 ± 20.45
48 h	100.00 ± 12.39	113.59 ± 27.11	119.37 ± 17.51	120.79 ± 15.00

Table 1. Metabolic activity (%) of *B. bassiana* after 24, 36, and 48 h of culture with λ-cyhalothrin, α-cypermethrin, and deltamethrin. Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

Element mg g^{-1} dry mass	Control	Pyrethroids (mg L^{-1})								
		λ-cyhalothrin			α-cypermethrin			Deltamethrin		
		5	50	100	5	50	100	5	50	100
Na	2.68 ± 0.11	2.54 ± 0.18	1.84 ± 0.18**	1.31 ± 0.10**	1.94 ± 0.24**	1.64 ± 0.02**	1.73 ± 0.03**	1.50 ± 0.18**	1.39 ± 0.27**	1.26 ± 0.22**
Mg	0.28 ± 0.02	0.19 ± 0.04**	0.27 ± 0.03	0.34 ± 0.03*	0.18 ± 0.01**	0.22 ± 0.00*	0.22 ± 0.01*	0.31 ± 0.00	0.21 ± 0.00**	0.26 ± 0.02
K	11.28 ± 0.03	11.71 ± 0.52	8.77 ± 0.53**	7.43 ± 0.25**	8.47 ± 0.37**	8.64 ± 0.55**	10.64 ± 0.91	11.32 ± 0.24	9.13 ± 0.06**	8.79 ± 0.61**
Ca	2.46 ± 0.06	2.45 ± 0.09	2.06 ± 0.10**	2.25 ± 0.14	2.15 ± 0.01**	2.12 ± 0.14**	2.26 ± 0.09	2.16 ± 0.04**	1.90 ± 0.11**	1.68 ± 0.15**

Table 2. Content of sodium, magnesium, potassium, and calcium in the *B. bassiana* cells after cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin. Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

of blastospores incubated for 24 and 48 h with pesticides at the concentrations of 50 and 100 mg L^{-1} (Supplementary Table S2). Among the tested insecticides, cyhalothrin had the most detrimental effect on this process. According to our knowledge, there are no data regarding the effect of pyrethroids on blastospores formation in liquid cultures. However, in the cultures on solid media, a decrease in the sporulation of *B. bassiana* was noted in the presence of cypermethrin and deltamethrin[22]. Inhibition of *B. bassiana* sporulation by herbicides in solid media has also been documented[23]. It seems that xenobiotics quite often inhibit the conidiation process, but the molecular mechanisms of this phenomenon are still unknown.

The study analyzed a wide range of insecticides concentrations, which were selected based on manufacturers' recommended doses for field applications. The lowest concentration tested in the study (5 mg L^{-1}) is tenfold lower than that recommended for commercial preparations for application per 100 m^2 of the area. The concentration 50 mg L^{-1} is within the recommended concentration range of cypermethrin and deltamethrin but exceeds that of cyhalothrin. The highest tested concentration (100 mg L^{-1}) is twice as high as the recommended concentration of cyhalothrin and deltamethrin. Because cypermethrin is used at high doses in the field, this concentration does not exceed the range of commonly applied range of doses.

The data reported in the literature are in line with our results. Pyrethroids used in recommended (and higher) doses strongly inhibited entomopathogenic fungi during the initial period of growth, limiting the rate of colony formation by more than 40%, while at lower doses these insecticides had a minimal inhibitory effect[24].

We also checked whether the highest tested insecticide concentration influenced the metabolic activity of entomopathogenic fungi as previous studies have only analyzed the effect of pyrethroids on their growth. The tests conducted in our study did not show any negative effect of pyrethroids on the metabolic activity of *B. bassiana*, and even a slight increase in activity was found in the samples exposed to the tested substances (Table 1). In 24-h cultures, a 1.47-fold increase in metabolic activity was noted in the presence of deltamethrin ($P=0.000176$), 1.36-fold increase in the presence of λ-cyhalothrin ($P=0.000237$), and 1.32-fold increase in the presence of α-cypermethrin ($P=0.000376$). In 36-h cultures, a 1.1-fold increase in metabolic activity was observed in the presence of λ-cyhalothrin ($P=0.016223$); however, in the presence of α-cypermethrin and deltamethrin, the metabolic activity in the samples was comparable to that observed in the control. In 48-h cultures, no differences in metabolic activity were found between the cultures exposed to pyrethroids and the biotic control.

Despite the growth inhibition, the tested insecticides did not adversely affect the metabolic activity of *B. bassiana*. This finding is different from the previous observations because most of the toxic compounds studied so far have been observed to cause a simultaneous reduction in the growth and viability of microorganisms. Różalska et al.[18] used the same method in their study to examine the metabolic activity of *Metarhizium robertsii*, another entomopathogenic fungal species. The authors found that the addition of both 4-*n*-nonylphenol and technical nonylphenol caused a statistically significant decrease in the metabolic activity of the tested strain.

Effect of pyrethroids on the content of sodium, potassium, calcium, and magnesium in biomass. All tested insecticides caused a decrease in sodium content in the biomass of *B. bassiana* (Table 2). The addition of 100 mg L^{-1} of λ-cyhalothrin and 50 and 100 mg L^{-1} of deltamethrin resulted in a two-fold reduction in the amount of this element in the fungal cells. To our knowledge, our study is the first to describe this

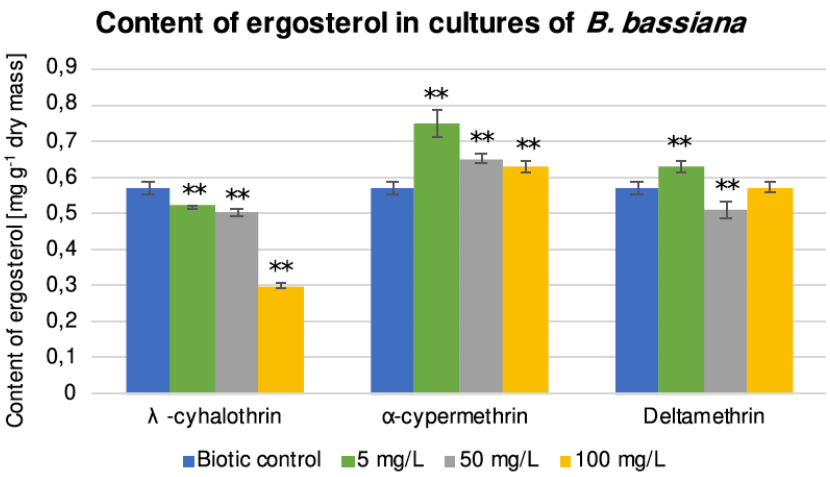

Figure 1. Content of ergosterol [mg g^{-1} dry mass] in the *B. bassiana* cells after 48 h of cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin. All samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

phenomenon for fungi. The insecticidal action of pyrethroids is based on their binding to sodium voltage-gated channels in the neuronal membranes of insects[25]. Interestingly, it has been postulated that genes coding sodium voltage-gated channels are not present in the fungal genomes[26] and the role of other sodium-transporting channels in fungi is still being elucidated.

The presence of pyrethroids was also found to influence the content of other elements in *B. bassiana* biomass (Table 2). Cypermethrin (even at low concentration) decreased the levels of K, Mg, and Ca, while deltamethrin caused a decrease in their levels in fungal cells at a concentration of 50 mg L^{-1}. Changes in the endogenous level of these elements might have resulted from increased membrane permeability after the exposure of fungal cells to pyrethroids.

A similar effect or leakage of potassium ions from the cells of *Candida albicans* associated with an increase in membrane permeability was observed by Lee and Lee (2014)[27] after the treatment of cells with curcumin. The results of their study indicated that pyrethroids cause disturbances in the ionic balance not only in insects, but also in entomopathogenic fungi. On the other hand, our results showed that pyrethroids did not affect the viability of *B. basiana*. Therefore, further research is needed to elucidate the mechanism that enables the fungi to remain viable in the presence of pyrethroids, despite the negative impact of these compounds on the integrity of the cell membrane.

Effect of pyrethroids on the ergosterol content in entomopathogenic fungi. The changes in the elemental content in fungal biomass may be associated with changes in the permeability of cell membranes. Ergosterol is an important marker of cell response to toxic substances. It plays a key role in many cell processes and is crucial for the integrity and functioning of the plasma membrane[20].

Our study showed that the studied pyrethroids differed in their effects on the ergosterol content of *B. bassiana* cells. λ-cyhalothrin, at all tested concentrations (5, 50, and 100 mg L^{-1}), caused a statistically significant decrease in content (by 8.78% ($P=0.000190$), 12.29% ($P=0.000163$), and 47.37% ($P=0.000163$), respectively), in comparison to the biotic control), while α-cypermethrin caused an increase in its synthesis in *B. bassiana* cells (Fig. 1). In the case of deltamethrin, an increase in the ergosterol amount was observed at the concentration of 5 mg L^{-1}, but at 50 mg L^{-1} a slight reduction in this component was observed in the fungal cells.

The literature has no information regarding the modifications in ergosterol level in the presence of pyrethroids in organisms, but the results observed in the content of elements and ergosterol in this work suggest that pyrethroids affect the cell membranes of *B. bassiana*.

Among the examined pyrethroids, the strongest membrane permeability in *B. bassiana* cells was found in presence of λ-cyhalothrin. Moreover, the lowest levels of ergosterol and higher phosphatidylcholine (PC)/phosphatidylethanolamine (PE) ratio were noticed in the cells in the presence of this insecticide. Considering the fact that ergosterol, PC, and PE regulate membrane permeability and fluidity, it seems that a decrease in the levels of ergosterol and PE caused an increase in the membrane permeability of fungal cells.

Effect of pyrethroids on phospholipid compositions. The most important phospholipid classes determined in the *B. bassiana* membranes are presented in Table 3. Quantitative lipidomic analyses revealed the highest content of PC (51.08%) and PE (45.48%) in the membranes. The contents of phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA) was estimated as 2.78%, 0.58%, and 0.08%, respectively. In a previous study that examined the phospholipid profile of *B. bassiana,* PC was identified as the dominant class of phospholipids, followed by PE, the amount of which was found to be half of that of PC[28]. Other phospholipid

Phospholipid class	Control	Pyrethroids (100 mg L^{-1})		
		λ-cyhalothrin	α-cypermethrin	Deltamethrin
PA relative abundance (%)	0.08 ± 0.03	0.06 ± 0.01	0.09 ± 0.03	0.07 ± 0.01
PC relative abundance (%)	51.08 ± 1.35	54.66 ± 0.37**	53.38 ± 0.92**	53.76 ± 0.57**
PE relative abundance (%)	45.48 ± 1.34	40.83 ± 0.74**	42.18 ± 0.94**	42.20 ± 0.51**
PI relative abundance (%)	2.78 ± 0.07	3.85 ± 0.48**	3.68 ± 0.32**	3.49 ± 0.19**
PS relative abundance (%)	0.58 ± 0.08	0.60 ± 0.10	0.66 ± 0.05	0.49 ± 0.09
PC/PE	1.12 ± 0.06	1.34 ± 0.03**	1.27 ± 0.05**	1.27 ± 0.03**
PI/PS	4.84 ± 0.83	6.58 ± 1.90	5.59 ± 0.89	7.37 ± 1.95*

Table 3. Phospholipid composition and PC/PE and PI/PS ratios determined in the *B. bassiana* cells after 48 h of cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin. Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

classes (PS, PI, and PA) were also detected, with the content of PA being the lowest, as was also observed in our work.

The lipidomic data of this study revealed that all tested pyrethroids affected the phospholipid profile of *B. bassiana* and caused a statistically significant increase in the PC content with a simultaneous decrease in the PE content (Table 3). As PC and PE are the main phospholipids in *B. bassiana* cells, the membrane dynamics of the cells depends on their ratio. PC is responsible for stabilizing the membrane bilayer phase, and maintaining its structure and functionality, while PE forms nonbilayer hexagonal phases. Changes in the phospholipid profile thus affect the properties of cell membranes[19]. The PC/PE ratio is also an essential factor of membrane integrity[29]. In this study, all the tested insecticides significantly increased this ratio ($P=0.000175$, $P=0.000201$, $P=0.000186$ in the presence of λ-cyhalothrin, α-cypermethrin, and deltamethrin, respectively), thereby leading to higher membrane fluidity. In addition, the increased PC/PE ratios were in concordance with the changes observed in the endogenous levels of K, Mg, and Ca.

The LC–MS/MS analysis revealed that PC 18:2 18:2, PC 18:2 18:1, and PE 16:0 18:2 were the major lipids in *B. bassiana* cells and that pyrethroids had a noticeable influence on their content (Supplementary Table S3). A statistically significant increase in the level of unsaturated PC 18:2 18:2 was noted in the presence of λ-cyhalothrin (34.49 ± 0.64%, $P=0.000232$), α-cypermethrin (33.85 ± 0.43%, $P=0.000238$), and deltamethrin (33.15 ± 0.39%, $P=0.000289$), compared to the biotic control (29.88 ± 0.25%). A higher amount of unsaturated PC shown by the analyses may also suggest an increase in membrane permeability. The level of PE 16:0 18:2, which was identified as the most predominant phospholipid, decreased to 18.04 ± 1.54% in the presence of λ-cyhalothrin ($P=0.005753$), 18.62 ± 2.44% in the presence of α-cypermethrin ($P=0.024558$), and 18.89 ± 1.30% in the presence of deltamethrin ($P=0.047449$), compared to the biotic control (21.34 ± 1.11%).

All tested pyrethroids also caused a statistically significant increase ($P<0.001$) in the PI content. The amount of PI 16:0 18:2 increased after the addition of λ-cyhalothrin ($P=0.005384$), and that of PI 18:2 18:2 increased in the presence of all tested pyrethroids ($P=0.000178$, $P=0.000181$, $P=0.000302$ with λ-cyhalothrin, α-cypermethrin, and deltamethrin, respectively) (Supplementary Table S3). PI acts as a precursor for the synthesis of many important lipid compounds, such as complex sphingolipids, phosphoinositides and inositol polyphosphates, and thus plays a key role in the basic metabolism. It is also essential for the anchoring of glycolipids in the plasma membrane[30]. Previous works have also demonstrated that toxic substances such as tributyltin caused an increase in PI content in fungal cell membranes[30]. No significant changes were observed in the content of PS (a precursor of PC and PE) and PA (a signal lipid) in this work.

Effect of pyrethroids on acylglycerols content in entomopathogenic fungi. Neutral lipids, TAGs and DAGs, are the main reserve material and important element of the phospholipids biosynthesis pathway[31]. The present study revealed that pyrethroids reduced the total amount of acylglycerols and the content of DAGs was higher than that of TAGs in *B. bassiana* cells (Fig. 2). The most significant reduction in TAGs was observed in the samples exposed to α-cypermethrin, while λ-cyhalothrin caused the greatest decrease in the content of DAGs. It has been found that the precursor of acylglycerols in the TAG biosynthesis pathway is glycerol-3-phosphate, which is converted to lysophosphatidic acid and subsequently to phosphatidic acid. In the next steps, phosphatidic acid is converted to DAG, and then DAG is converted to TAG[32]. No data can be found in the literature regarding the inhibition of acylglycerol synthesis by pyrethroids in entomopathogenic fungi. Moreover, in *Donax trunculus* clam, a reverse effect has been observed after the exposure of the organisms to pyrethroids[33].

Using LC–MS/MS, 13 TAG and 9 DAG species were identified in the *B. bassiana* samples (Supplementary Table S4). The major molecular TAGs detected were: 52:2, 54:3, 54:4, and 54:6 and DAGs were 34:2, 36:1, and 36:2. In the presence of pyrethroids, some changes in the content of individual neutral lipids were recorded. λ-Cyhalothrin caused a statistically significant decrease in the content of TAGs 52:4 ($P=0.041826$), 54:1 ($P=0.000199$), 54:2 ($P=0.000199$), 54:3 ($P=0.002386$), 54:4 ($P=0.000203$), and 54:6 ($P=0.000338$) and an increase in TAGs 48:0 ($P=0.000199$), 50:0 ($P=0.000199$), 52:1 ($P=0.000199$), and 52:2 ($P=0.000239$). α-Cypermethrin caused a statistically significant decrease in the content of TAGs 52:3 ($P=0.000417$), 52:4 ($P=0.018714$), 54:1 ($P=0.000199$), 54:2 ($P=0.000199$), 54:3 ($P=0.000250$), 54:4 ($P=0.002145$), and 54:6 ($P=0.000241$) with a simultaneous increase in the content of TAGs 48:0 ($P=0.000199$), 50:0 ($P=0.000199$),

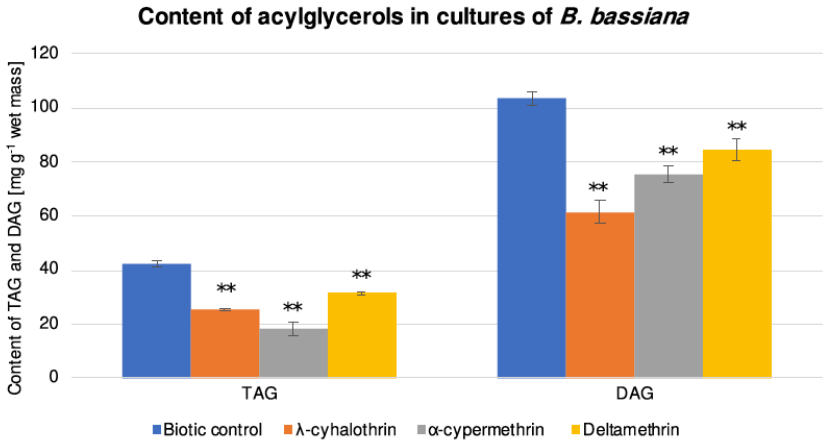

Figure 2. Content of tri- and diacylglycerols in the *B. bassiana* cells after 48 h of cultivation with λ-cyhalothrin, α-cypermethrin and deltamethrin at a concentration of 100 mg L^{-1}. All samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

	Control	Pyrethroids (100 mg L^{-1})		
		λ-cyhalothrin	α-cypermethrin	Deltamethrin
Membrane permeability [%]	100.00 ± 5.77	123.51 ± 4.49**	115.69 ± 4.10*	107.00 ± 1.80
Recovery [%]				
Culture medium	–	1.93 ± 0.24	2.50 ± 0.12	1.66 ± 0.33
Mycelium	–	71.84 ± 1.12	70.16 ± 0.83	99.64 ± 0.74

Table 4. Membrane permeability and accumulation of pyrethroids in the *B. bassiana* cells. Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

and 52:1 ($P=0.000199$). Our results indicate that deltamethrin had the weakest effect on individual TAG species and some changes were only recorded in the content of TAGs 54:0 ($P=0.000270$) and 54:2 ($P=0.000199$) and DAG 36:1 ($P=0.033229$).

λ-Cyhalothrin and α-cypermethrin increased the amounts of DAGs 32:0 ($P=0.000199$; $P=0.009261$), and 34:2 ($P=0.000278$; $P=0.000199$); however, a higher content of DAG 34:0 ($P=0.005059$) was also noticed in the *B. bassiana* samples cultivated with λ-cyhalothrin. Interestingly, the abundance of DAG 36:1 was found to be clearly reduced in the presence of λ-cyhalothrin ($P=0.000210$) and α-cypermethrin ($P=0.000254$), while deltamethrin caused an increase in the content of this acylglycerol ($P=0.033229$).

Our results suggest that λ-cyhalothrin and α-cypermethrin increased the content of saturated TAGs and DAGs and decreased the content of unsaturated TAGs. Deltamethrin had a slight effect on the amounts of individual TAGs and DAGs.

Compared to the control, the lowest TAG/DAG ratio was found in the presence of cypermethrin ($P=0.003244$), which may indicate increased accumulation of DAG related to TAG inhibition (Supplementary Table S4).

TAGs are the basic reserve material for cells and together with DAG they constitute an important element of phospholipid biosynthesis[19,34] and also play a crucial role in protecting cells against oxidative damage[35]. Studies in mammals showed that accumulation of TAGs in lipid droplets prevents oxidative stress in cells by limiting the generation of reactive oxygen species (ROS)[35]. On the other hand, in this study, the fungal cells exposed to pyrethroids had a lower accumulation of TAGs accompanied by an increased percentage of hyphae containing superoxide anion radicals and hydrogen peroxide.

TAGs are also involved in fungal virulence, supporting the penetration of fungi into the insect cuticle[29,34]. This suggests that pyrethroids affect the metabolism of fungi, which play an important ecological role in the environment, controlling the insect populations.

Effect of pyrethroids on membrane permeability and their accumulation. The membrane permeability of *B. bassiana* cells exposed to pyrethroids was determined by intracellular accumulation of propidium iodide (Table 4). The results showed a statistically significant increase in red fluorescence in fungal biomass incubated in the presence of λ-cyhalothrin and α-cypermethrin at the concentration of 100 mg L^{-1}

			Pyrethroids (100 mg L^{-1})		
		Control	λ-cyhalothrin	α-cypermethrin	Deltamethrin
O_2^{-}	24 h	1.76 ± 1.26	1.93 ± 1.02	4.09 ± 2.42*	2.37 ± 0.74
	36 h	0.55 ± 0.08	4.73 ± 0.82**	10.97 ± 4.15**	7.34 ± 1.44**
	48 h	1.51 ± 1.02	7.87 ± 3.25*	18.62 ± 3.22**	22.80 ± 5.31**
H_2O_2	24 h	nd	nd	nd	nd
	36 h	0.14 ± 0.00	0.22 ± 0.06	0.63 ± 0.31**	0.37 ± 0.12
	48 h	0.33 ± 0.26	3.80 ± 0.59**	4.64 ± 2.00**	3.28 ± 0.99**

Table 5. Percentage of hyphae containing superoxide anion radicals and hydrogen peroxide in *B. bassiana* after incubation with λ-cyhalothrin, α-cypermethrin, and deltamethrin. Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P < 0.01$, $^{*}P < 0.05$. nd - not detected.

(by 23.5%, $P = 0.000196$ and 15.7%, $P = 0.000328$, respectively) and a slight increase in the cultures containing deltamethrin at the concentration of 100 mg L^{-1} (7%). A statistically significant increase in the PC/PE ratio was also confirmed (Table 3). Furthermore, a higher content of ergosterol was noted in the samples supplemented with α-cypermethrin and deltamethrin, which proved an increase in the fluidity of *B. bassiana* cell membranes (Fig. 1).

Previous studies have also reported the changes in the fungal membranes permeability caused by pesticides. Higher membrane permeability associated with an increase in the PC/PE ratio was described for several strains of *Trichoderma* spp. cultured with chloroacetanilide herbicides[21]. Similarly, increased membrane fluidity was observed for the herbicide degrader *Umbelopsis isabellina* in the presence of 2,4-D[36]. Increased membrane fluidity was found to facilitate the transport of toxic substances into the fungal cells, which causes the accumulation or even degradation of these substances[7].

In this study, all tested pyrethroids were found to be accumulated in the mycelium of *B. bassiana*. The amounts of λ-cyhalothrin and α-cypermethrin in fungal biomass were estimated at 71.84% and 70.16%, respectively, while deltamethrin was accumulated at 99.64%. This suggests that increased membrane permeability promotes the penetration of pyrethroids into the mycellium.

Effect of pyrethroids on ROS production. Literature data indicate that the mechanism of toxicity of insecticides could be associated with enhanced intracellular ROS production/antioxidant enzyme activity[8]. Therefore, in this study, we determined the intracellular level of superoxide anion radicals and hydrogen peroxide in *B. bassiana* mycelia following exposure to pyrethroids (Table 5).

At the beginning of the experiment (24-h incubation), a slight increase in the level of superoxide anion radicals was observed only in the samples cultivated with α -cypermethrin, while H_2O_2 was not detected in the biomass. It has been found that under stress conditions, superoxide anion radicals appear first and are then converted by superoxide dismutase to hydrogen peroxide. In the present study, prolonging the incubation time resulted in a significant increase in the ROS levels. In the 36-h biomass, the amount of superoxide anion radicals increased by eightfold, almost 20-fold and 13-fold in the presence of λ-cyhalothrin ($P = 0.008298$), α-cypermethrin ($P = 0.000161$), and deltamethrin ($P = 0.000194$), respectively. Similarly, in 48-h cultures, approximately fivefold ($P = 0.011729$), 12-fold ($P = 0.000175$), and 15-fold ($P = 0.000175$) increase in the level of O_2^{-} were noted. A statistically significant increase in hydrogen peroxide by 11-fold, 14-fold, and ninefold was detected at 48 h of incubation in the samples exposed to λ-cyhalothrin ($P = 0.000231$), α-cypermethrin ($P = 0.000177$), and deltamethrin ($P = 0.000585$), respectively. These results clearly highlight that oxidative stress was induced in *B. bassiana* after the exposure to pyrethroids. Oxidative stress may have been caused by the entry and accumulation of pyrethroids in mycelium, which, as mentioned earlier, may be correlated with the increase in membrane permeability. Furthermore, the observed decrease of TAGs in the presence of pyrethroids may influence the generation of free radicals, the level of which may also depend on the amount of TAGs in cells.

Due to their lifestyle, entomopathogenic fungi may often experience oxidative stress by different factors, including exposure to insect host defense such as production of ROS[37]. Therefore, they are characterized by extremely flexible metabolism which enables them to grow under various environmental conditions. The results of our work confirmed that pyrethroids might be one of the harmful factors, which is in line with the observation made so far in fish and rats that oxidative stress is induced by these substances[38,39].

Effect of pyrethroids on secondary metabolite production by *Beauveria* species. Oosporein, a secondary metabolite belonging to the octadepsipeptide group, exhibits insecticidal, antiviral, and antibacterial effects[40]. It is an important metabolite of entomopathogenic fungi and stimulates infection in insects[7]. In our study, a lack of oosporein production was observed in the 24-h culture and the presence of pyrethroids was found to inhibit the pigment production even in the later period of incubation. In 36-h cultures, the content of oosporein was decreased in the samples exposed to pyrethroids than in the biotic control (λ-cyhalothrin: 6.7-fold ($P = 0.000254$), α-cypermethrin: 11.3-fold ($P = 0.000242$), deltamethrin: 7.5-fold ($P = 0.000250$)) (Fig. 3). In the 48-h culture, a slight increase in pigment content was observed in the samples with all tested insecticides, but these differences were not statistically significant (Fig. 3).

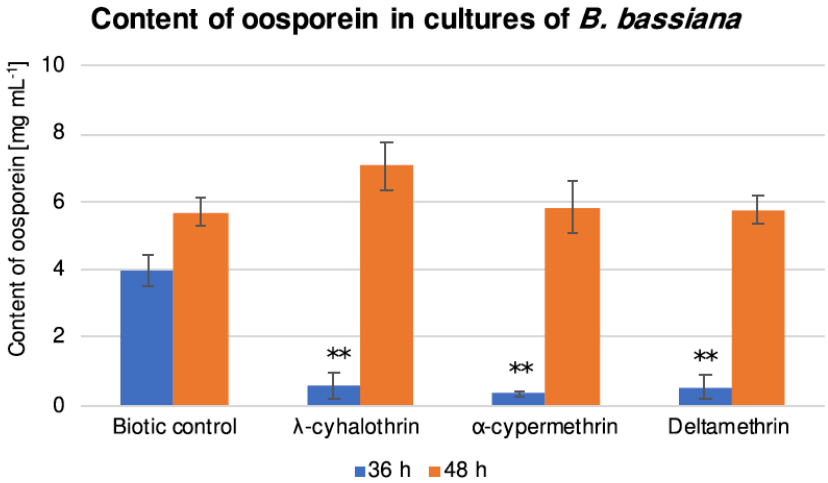

Figure 3. Content of oosporein [mg mL^{-1}] in *B. bassiana* after 36 and 48 h of cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin at a concentration of 100 mg L^{-1}. All samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: $^{**}P<0.01$, $^{*}P<0.05$.

In general, microorganisms produce secondary metabolites to protect them against both biotic and abiotic stress[41]. The influence of toxic compounds on the production of these metabolites has been poorly understood, and so far only the inhibition of destruxins production by *Metarhizium* sp. by acetamiprid has been proved[42]. Temporary inhibition of oosporein production in the presence of pyrethroids indicates a harmful effect of these substances on entomopathogenic fungi and could be an effect of both slight growth inhibition and induction of oxidative stress, which could reduce the infectious potential of the fungi and thus adversely affect their behavior in the environment.

Conclusions

Entomopathogenic fungi and pyrethroids do not only coexist in the environment but can also be applied together in pest control, and thus it seems highly important to understand the relationship between them. The findings of our study support the hypothesis that pyrethroids influence the metabolism of *B. bassiana* cells, especially the membrane lipid profile and permeability. By increasing the membrane permeability, pyrethroids gain access into the fungal cells and accumulate within them, leading to the formation of free radicals and reduction of sodium content. Intriguingly, these pesticides were found to cause a decrease in the level of sodium and temporarily lower the amount of the secondary metabolite oosporein, which suggests that their influence on cellular metabolism is very complex. For effective application of these two plant protection agents in pest control, understanding the mechanisms by which pyrethroids affect the metabolism of entomopathogenic fungi is critical, but the obtained results suggest that further research using tools such as proteomics or metabolomics is needed to fully uncover these phenomena.

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References

1. Antwi, F. B. & Reddy, G. V. P. Toxicological effects of pyrethroids on non-target aquatic insects. *Environ. Toxicol. Pharmacol.* **40**, 915–923 (2015).
2. Cycoń, M. & Piotrowska-Seget, Z. Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review. *Front. Microbiol.* 7, 1–26 (2016).
3. Skolarczyk, J., Pekar, J. & Nieradko-Iwanicka, B. Zaburzenia immunologiczne wywołane narażeniem na insektycydy z grupy pyretroidów. *Postepy Hig. Med. Dosw.* **71**, 446–453 (2017).
4. Tang, W. *et al.* Pyrethroid pesticide residues in the global environment: an overview. *Chemosphere* **191**, 990–1007 (2018).
5. Milner, R. J. Selection and characterization of strains of Metarhizium anisopliae for control of soil insects in Australia. In *Biological Control of Locusts and Grasshoppers* 200–207 (1991).
6. Mascarin, G. M. & Jaronski, S. T. The production and uses of Beauveria bassiana as a microbial insecticide. *World J. Microbiol. Biotechnol.* **32**, 1–26 (2016).
7. Litwin, A., Nowak, M. & Różalska, S. Entomopathogenic fungi: unconventional applications. *Rev. Environ. Sci. Biotechnol.* **19**, 23–42 (2020).
8. Forlani, L., Juárez, M. P., Lavarías, S. & Pedrini, N. Toxicological and biochemical response of the entomopathogenic fungus Beauveria bassiana after exposure to deltamethrin. *Pest Manag. Sci.* **70**, 751–756 (2014).
9. Joshi, M., Gaur, N. & Pandey, R. Compatibility of entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae with selective pesticides. *J. Entomol. Zool. Stud.* **6**, 867–872 (2018).
10. Abidin, A. F., Ekowati, N. & Ratnaningtyas, N. I. Insecticide compatibility to the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae. *Scr. Biol.* **4**, 273–279 (2017).

11. Chen, X.-M., Wang, X.-Y., Lu, W. & Zheng, X.-L. Use of Beauveria bassiana in combination with commercial insecticides to manage Phauda flammans (Walker) (Lepidoptera: Phaudidae): testing for compatibility and synergy. *J. Asia. Pac. Entomol.* **24**, 272–278 (2021).
12. Tkaczuk, C., Harasimiuk, M., Król, A. & Beres, P. K. The effect of selected pesticides on the growth of entomopathogenic fungi Hirsutella nodulosa and Beauveria bassiana. *J. Ecol. Eng.* **16**, 177–183 (2015).
13. Feng, M.-G., Johnson, J. B. & Kish, L. P. Survey of Entomopathogenic fungi naturally infecting cereal aphids (Homoptera: Aphididae) of irrigated grain crops in Southwestern Idaho. *Environ. Entomol.* **19**, 1534–1542 (1990).
14. Puterka, G. J. Fungal pathogens for arthropod pest control in orchard systems: mycoinsecticidal approach for pear psylla control. *Biocontrol* **44**, 183–209 (1999).
15. Xiao, G. *et al.* Genomic perspectives on the evolution of fungal entomopathogenicity in Beauveria bassiana. *Sci. Rep.* **2**, 59 (2012).
16. Valero-Jiménez, C. A. *et al.* Comparative genomics of Beauveria bassiana: Uncovering signatures of virulence against mosquitoes. *BMC Genom.* **17**, 1–12 (2016).
17. Li, S., Yi, W., Chen, S. & Wang, C. Empirical support for the pattern of competitive exclusion between insect parasitic fungi. *J. Fungi* **7**, 189 (2021).
18. Różalska, S., Glińska, S. & Długoński, J. Metarhizium robertsii morphological flexibility during nonylphenol removal. *Int. Biodeterior. Biodegrad.* **95**, 285–293 (2014).
19. Stolarek, P., Różalska, S. & Bernat, P. Lipidomic adaptations of the Metarhizium robertsii strain in response to the presence of butyltin compounds. *Biochim. Biophys. Acta - Biomembr.* **1861**, 316–326 (2019).
20. Suchodolski, J., Muraszko, J., Bernat, P. & Krasowska, A. A crucial role for ergosterol in plasma membrane composition, localisation, and activity of Cdr1p and H+-ATPase in Candida albicans. *Microorganisms* **7**, 1–17 (2019).
21. Nykiel-Szymańska, J., Różalska, S., Bernat, P. & Słaba, M. Assessment of oxidative stress and phospholipids alterations in chloroacetanilides-degrading Trichoderma spp. *Ecotoxicol. Environ. Saf.* **1861**, 316–326 (2019).
22. de Oliveira, C. N., Neves, P. M. O. J. & Kawazoe, L. S. Compatibility between the entomopathogenic fungus beauveria bassiana and insecticides used in coffee plantations. *Sci. Agric.* **60**, 663–667 (2003).
23. Kos, K. & Celar, F. A. Sensitivity of the entomopathogenic fungus Beauveria bassiana (Bals.-Criv.) Vuill. to selected herbicides. *Pest. Manag. Sci.* **69**, 717–721 (2013).
24. Tkaczuk, C., Majchrowska-Safaryan, A. & Zawadzka, M. The effect of Spinosad and selected synthetic insecticides on the growth of entomopathogenic fungi. *J. Res. Appl. Agric. Eng.* **58**, 194–198 (2013).
25. Soderlund, D. M. Molecular mechanisms of pyrethroid insecticide neurotoxicity: Recent advances. *Arch. Toxicol.* **86**, 165–181 (2012).
26. Liebeskind, B. J., Hillis, D. M. & Zakon, H. H. Evolution of sodium channels predates the origin of nervous systems in animals. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 9154–9159 (2011).
27. Lee, W. & Lee, D. G. An antifungal mechanism of curcumin lies in membrane-targeted action within Candida albicans. *IUBMB Life* **66**, 780–785 (2014).
28. Palma-Guerrero, J. *et al.* Membrane fluidity determines sensitivity of filamentous fungi to chitosan. *Mol. Microbiol.* **75**, 1021–1032 (2010).
29. Chen, Y. *et al.* Diverse effect of phosphatidylcholine biosynthetic genes on phospholipid homeostasis, cell autophagy and fungal developments in Metarhizium robertsii. *Environ. Microbiol.* **20**, 293–304 (2018).
30. Bernat, P., Gajewska, E., Szewczyk, R., Słaba, M. & Długoński, J. Tributyltin (TBT) induces oxidative stress and modifies lipid profile in the filamentous fungus Cunninghamella elegans. *Environ. Sci. Pollut. Res.* **21**, 4228–4235 (2014).
31. Czabany, T., Athenstaedt, K. & Daum, G. Synthesis, storage and degradation of neutral lipids in yeast. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids* **1771**, 299–309 (2009).
32. Keyhani, N. O. Lipid biology in fungal stress and virulence: entomopathogenic fungi. *Fungal Biol.* **122**, 420–429 (2018).
33. Aouini, F. *et al.* Short term exposure to permethrin alters lipid classes and fatty acid composition of the unfed clam Donax trunculus from Tunisian coast (Southern Mediterranean). *Cah. Biol. Mar.* **58**, 163–172 (2017).
34. Gao, Q. *et al.* Phospholipid homeostasis maintains cell polarity, development and virulence in Metarhizium robertsii. *Environ. Microbiol.* **18**, 3976–3990 (2016).
35. Fan, J., Yu, L. & Xu, C. A central role for triacylglycerol in membrane lipid breakdown, fatty acid β-oxidation, and plant survival under extended darkness. *Plant Physiol.* **174**, 1517–1530 (2017).
36. Bernat, P., Nykiel-Szymańska, J., Stolarek, P., Słaba, M. & Różalska, S. 2,4- dichlorophenoxyacetic acid-induced oxidative stress: metabolome and membrane modifications in Umbelopsis isabellina, a herbicide degrader. *PLoS One* **13**, 1002 (2018).
37. Lovett, B. & St. Leger, R. J. Stress is the rule rather than the exception for Metarhizium. *Curr. Genet.* **61**, 253–261 (2015).
38. Yang, C., Lim, W. & Song, G. Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **234**, 10096 (2020).
39. Kale, M., Rathore, N., John, S. & Bhatnagar, D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol. Lett.* **105**, 197–205 (1999).
40. Feng, P., Shang, Y., Cen, K. & Wang, C. Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. *Proc. Natl. Acad. Sci.* **112**, 11365–11370 (2015).
41. Mc Namara, L. *et al.* Oosporein, an abundant metabolite in Beauveria caledonica, with a feedback induction mechanism and a role in insect virulence. *Fungal Biol.* **123**, 601–610 (2019).
42. Nowak, M., Bernat, P., Mrozińska, J. & Rózalska, S. Acetamiprid affects destruxins production but its accumulation in Metarhizium sp. spores increases infection ability of fungi. *Toxins (Basel).* **12**, 5903 (2020).

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Author contributions
Conceptualization (S.R.), methodology (A.L., P.B, M.N., M.S), validation (A.L., P.B, M.N., M.S), investigation (A.L., P.B, M.N., M.S), writing (S.R., A.L.), supervision (S.R.), founding acquisition and project administration (S.R.).

Competing interests
The authors declare no competing interests.

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Artykuł naukowy P2 – *Supplementary*

Lipidomic response of the entomopathogenic fungus *Beauveria bassiana* to pyrethroids

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Supplementary Table S1. Dry biomass (g L^{-1}) of *B. bassiana* after 36 and 48 h of cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin.

	Pyrethroids (mg L^{-1})									
		λ –cyhalothrin			α –cypermethrin			Deltamethrin		
	Control	5	50	100	5	50	100	5	50	100
36 h	1.67 ± 0.14	1.75 ± 0.01	1.20 ± 0.23**	1.25 ± 0.03**	1.77 ± 0.01	1.26 ± 0.16**	1.34 ± 0.02**	1.71 ± 0.15	1.42 ± 0.07**	1.31 ± 0.05**
48 h	3.78 ± 0.21	3.93 ± 0.04	3.67 ± 0.06	3.19 ± 0.08**	4.03 ± 0.17	3.76 ± 0.11	3.29 ± 0.71*	3.89 ± 0.11	3.66 ± 0.09	3.56 ± 0.14

Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: ** – $p < 0.01$, * – $p < 0.05$.

Supplementary Table S2. Number of blastospores (expressed as 10^7) in *B. bassiana* cultures supplemented with pyrethroids after 24 and 48 h of incubation.

	Pyrethroids (mg L^{-1})									
		λ -cyhalothrin			α -cypermethrin			Deltamethrin		
	Control	5	50	100	5	50	100	5	50	100
24 h	1.045 ± 0.46	0.989 ± 0.27	0.630 ± 0.22**	0.295 ± 0.10**	1.054 ± 0.36	0.692 ± 0.4**	0.322 ± 0.15**	1.008 ± 0.46	0.682 ± 0.17**	0.264 ± 0.10**
48h	7.453 ± 1.39	8.134 ± 2.61	1.597 ± 0.40**	0.856 ± 0.26**	7.55 ± 0.72	6.489 ± 1.58	4.436 ± 1.24**	7.768 ± 2.73	6.089 ± 0.84**	3.922 ± 0.90**

Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: ** – $p < 0.01$, * – $p < 0.05$.

Supplementary Table S3. Phospholipid species composition determined in the *B. bassiana* cells after 48 h of cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin.

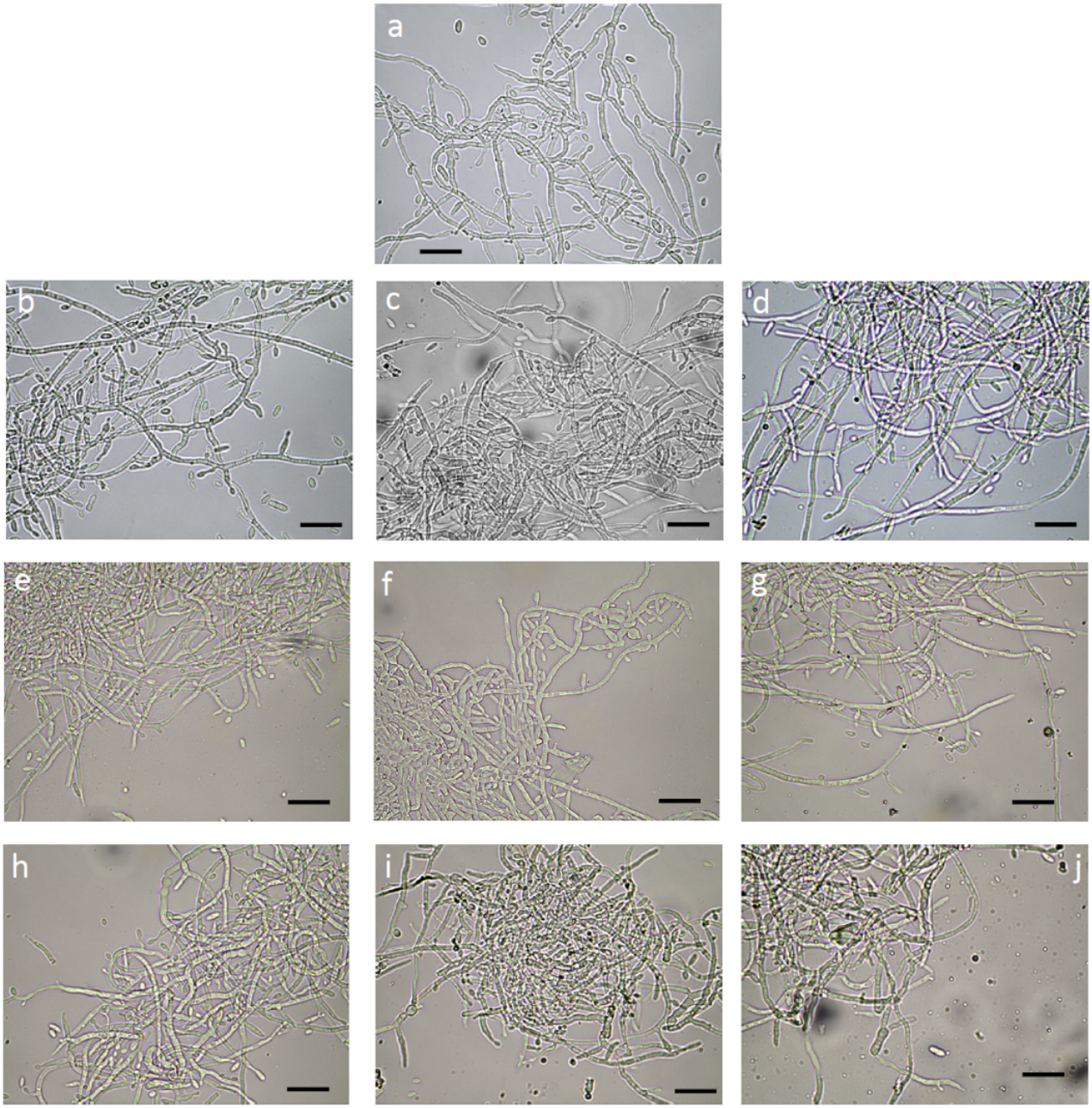
Phospholipid speciess relative abundance (%)	Pyrethroids (mg L^{-1})			
	Control	λ –cyhalothrin	α –cypermethrin	Deltamethrin
PA 16:0 18:2	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
PA 18:2 18:2	0.05 ± 0.01	0.03 ± 0.02	0.07 ± 0.02	0.04 ± 0.01
PC 16:0 18:2	3.07 ± 0.06	3.62 ± 0.22**	4.16 ± 0.11**	3.92 ± 0.03**
PC 18:3 18:3	0.11 ± 0.01	0.16 ± 0.02**	0.18 ± 0.02**	0.17 ± 0.02**
PC 18:3 18:2	2.23 ± 0.04	2.45 ± 0.21*	2.68 ± 0.13**	2.53 ± 0.08**
PC 18:3 18:1	0.48 ± 0.10	0.49 ± 0.04	0.38 ± 0.03*	0.47 ± 0.04
PC 18:2 18:2	29.88 ± 0.25	34.49 ± 0.64**	33.85 ± 0.43**	33.15 ± 0.39**
PC 18:2 18:1	12.79 ± 0.72	11.58 ± 0.68**	10.61 ± 0.47**	11.67 ± 0.71*
PC 18:1 18:1	2.52 ± 0.36	1.87 ± 0.25**	1.52 ± 0.05**	1.84 ± 0.15**
PE 16:0 18:2	21.34 ± 1.11	18.04 ± 1.54**	18.62 ± 2.44*	18.89 ± 1.30*
PE 18:3 18:2	0.75 ± 0.05	0.80 ± 0.14	1.00 ± 0.06**	0.91 ± 0.04*
PE 18:2 18:2	8.19 ± 0.64	8.78 ± 0.35	11.34 ± 0.79**	10.35 ± 0.74**
PE 18:2 18:1	3.32 ± 0.14	3.55 ± 0.46	3.24 ± 0.33	3.28 ± 0.31
PE 18:1 18:1	2.61 ± 0.23	2.33 ± 0.19*	1.68 ± 0.14**	2.04 ± 0.14**
PE 16:0 18:1	7.22 ± 0.35	5.26 ± 0.41**	4.07 ± 0.25**	4.88 ± 0.48**
PE 18:2 18:0	2.05 ± 0.08	2.07 ± 0.08	2.23 ± 0.08	1.85 ± 0.28
PI 16:0 18:2	1.68 ± 0.06	2.02 ± 0.15**	1.90 ± 0.29	1.83 ± 0.10
PI 18:1 18:3	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
PI 18:2 18:2	0.94 ± 0.01	1.59 ± 0.31**	1.56 ± 0.17**	1.45 ± 0.09**
PI 18:1 18:2	0.16 ± 0.01	0.24 ± 0.03**	0.21 ± 0.02**	0.21 ± 0.01**
PS 16:0 18:2	0.31 ± 0.01	0.33 ± 0.02	0.32 ± 0.03	0.32 ± 0.01
PS 18:2 18:2	0.24 ± 0.08	0.24 ± 0.09	0.30 ± 0.02	0.13 ± 0.00
PS 18:1 18:2	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01

Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: ** – $p < 0.01$, * – $p < 0.05$.

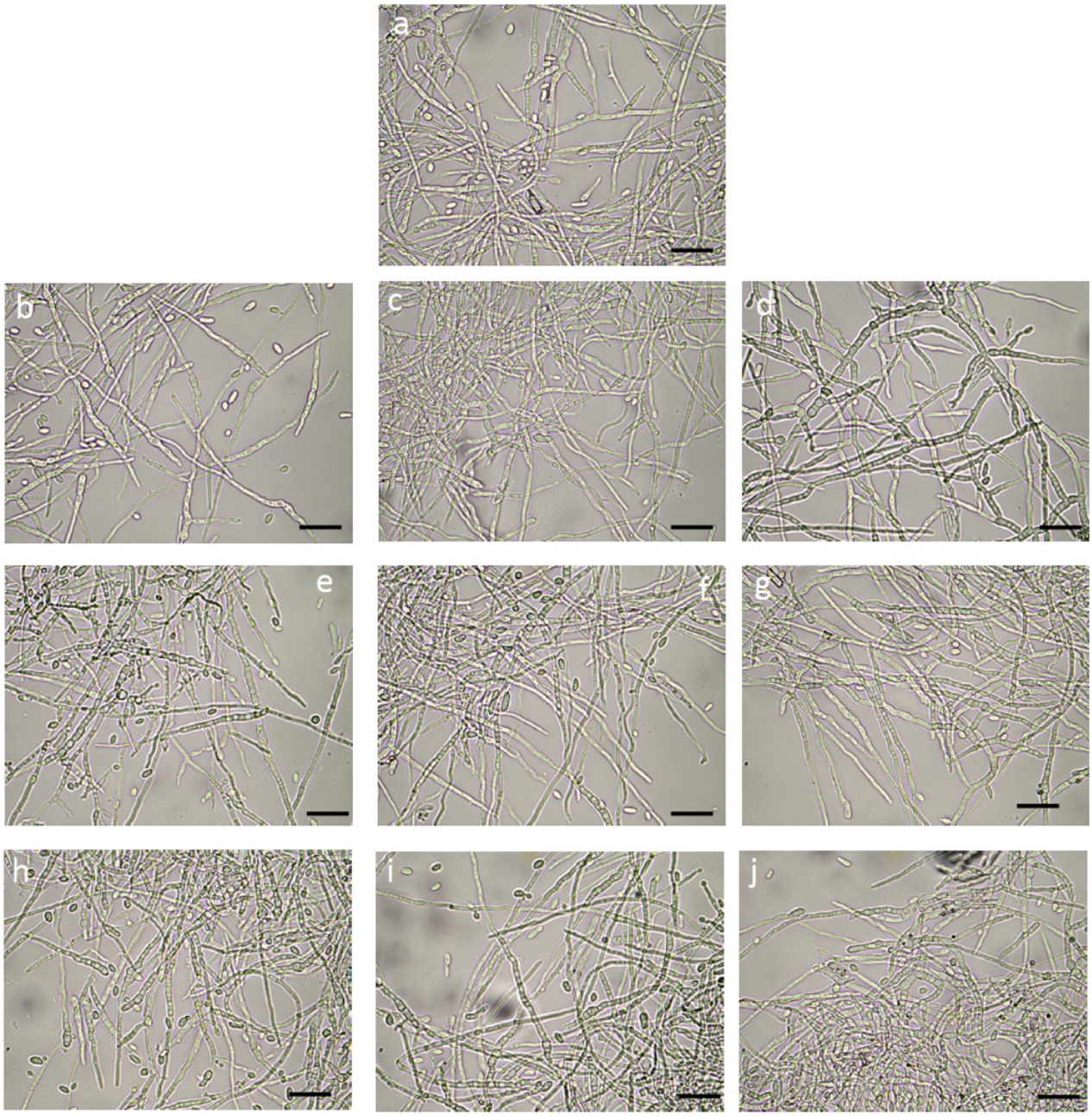
Supplementary Table S4. Percentage content of tri- and diacylglycerols and TAG/DAG ratio in the *B. bassiana* cells after 48 h of cultivation with λ-cyhalothrin, α-cypermethrin, and deltamethrin.

	Pyrethroids (mg L^{-1})			
	Control	λ –cyhalothrin	α –cypermethrin	Deltamethrin
TAG 48:0	3.54 ± 0.27	10.08 ± 0.29**	11.65 ± 0.21**	4.05 ± 0.68
TAG 50:0	1.16 ± 0.04	2.80 ± 0.12**	3.39 ± 0.31**	1.21 ± 0.01
TAG 50:1	6.21 ± 0.49	5.70 ± 0.22	6.60 ± 0.01	6.25 ± 0.58
TAG 52:1	3.23 ± 0.05	7.14 ± 0.46**	8.45 ± 0.84**	3.52 ± 0.41
TAG 52:2	14.37 ± 0.70	17.30 ± 0.17**	15.24 ± 0.75	14.51 ± 0.46
TAG 52:3	10.21 ± 0.17	10.74 ± 0.87	8.29 ± 0.08**	10.46 ± 0.01
TAG 52:4	8.15 ± 0.51	7.42 ± 0.11*	7.31 ± 0.39*	8.06 ± 0.17
TAG 54:0	1.46 ± 0.06	1.55 ± 0.05	1.51 ± 0.15	1.91 ± 0.06**
TAG 54:1	3.63 ± 0.11	1.35 ± 0.35**	1.10 ± 0.06**	3.63 ± 0.20
TAG 54:2	5.07 ± 0.33	2.16 ± 0.01**	1.98 ± 0.17**	3.44 ± 0.57**
TAG 54:3	16.17 ± 0.97	13.68 ± 0.84**	12.44 ± 0.36**	16.98 ± 0.64
TAG 54:4	16.27 ± 0.93	11.25 ± 0.12**	13.43 ± 1.18**	15.29 ± 0.68
TAG 54:6	10.52 ± 0.26	8.83 ± 0.10**	8.61 ± 0.44**	10.70 ± 0.53
DAG 32:0	3.29 ± 0.26	9.96 ± 1.44**	5.43 ± 0.47**	1.72 ± 0.01
DAG34:0	2.08 ± 0.01	2.97 ± 0.48**	2.63 ± 0.22	2.46 ± 0.25
DAG34:1	1.75 ± 0.04	2.05 ± 0.56	2.16 ± 0.31	1.98 ± 0.19
DAG34:2	12.32 ± 0.37	15.00 ± 0.11**	16.99 ± 0.01**	12.30 ± 1.04
DAG36	3.54 ± 0.42	2.74 ± 1.34	4.26 ± 1.43	4.35 ± 1.21
DAG36:1	34.12 ± 1.59	21.60 ± 3.90**	23.00 ± 1.44**	39.17 ± 0.26*
DAG36:2	36.93 ± 2.67	40.07 ± 4.58	40.36 ± 1.37	33.66 ± 1.28
DAG36:3	4.97 ± 0.71	4.64 ± 1.00	4.32 ± 0.77	3.57 ± 0.87
DAG36:4	1.00 ± 0.12	0.96 ± 0.62	0.84 ± 0.35	0.80 ± 0.26
TAG/DAG	0.41 ± 0.00	0.41 ± 0.02	0.24 ± 0.02**	0.37 ± 0.02

Data are means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice. Results were tested by one-way ANOVA; significance: ** – $p < 0.01$, * – $p < 0.05$.



Supplementary Figure S1. Morphology of *B. bassiana* in 24 h cultures supplemented with pyrethroids. Control without toxic substances (a); λ-cyhalothrin at concentrations of 5, 50 and 100 mg L^{-1} (b-d, respectively), α-cypermethrin at concentrations of 5, 50 and 100 mg L^{-1} (e-g, respectively) and deltamethrin at concentrations of 5, 50 and 100 mg L^{-1} (h-j, respectively).
The scale bar represents 20 μm.



Supplementary Figure S2. Morphology of *B. bassiana* in 48 h cultures supplemented with pyrethroids. Control without toxic substances (a); λ-cyhalothrin at concentrations of 5, 50 and 100 mg L^{-1} (b-d, respectively), α-cypermethrin at concentrations of 5, 50 and 100 mg L^{-1} (e-g, respectively) and deltamethrin at concentrations of 5, 50 and 100 mg L^{-1} (h-j, respectively).
The scale bar represents 20 μm.

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Accumulation of pyrethroids induces changes in metabolism of the entomopathogenic fungus *Beauveria bassiana*—Proteomic and lipidomic background

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ABSTRACT

Advances in the agrochemical industry, such as using plant protection products e.g. pyrethroid insecticides, lead to environmental pollution via the accumulation of toxic compounds in soil. An interesting approach to overcoming this threat is using biopreparations based on entomopathogenic fungi that come into contact with the residues of the insecticides in the environment. The aim of this study was to determine whether the soil-dwelling entomopathogenic fungus *Beauveria bassiana* ARSEF 2860 is capable of accumulating pyrethroids (λ-cyhalothrin, α-cypermethrin and deltamethrin) and to identify the metabolomics and proteomic implications of this process. In this work, we demonstrated for the first time that the tested fungus accumulated pyrethroids as early as on day 2 of incubation with an average efficiency of 90%. Pyrethroids accumulated in large quantities in the mycelium of *B. bassiana* induced oxidative stress and interacted differently with the enzymes of the basic metabolic pathways, enzymes associated with the organization of the actin cytoskeleton and cell walls, as well as extracellular enzymes responsible for the infectious abilities (α-cypermethrin caused a 61% decrease in PR1, λ-cyhalothrin - a 31% decrease in PR2, which are proteolytic enzymes with a confirmed role in the infectious process). This study also revealed that the accumulated pyrethroids decreased the activity of phospholipase C, which increased the triacylglycerols/diacylglycerols (TAG/DAG) ratio, especially in mycelium in which α-cypermethrin was accumulated. It should be emphasized that the accumulation of pyrethroids in the environment is not fully understood, and current research suggests that entomopathogenic fungi may be part of the process.

Abbreviations: **ADP**, (adenosine diphosphate); **ATP**, (adenosine triphosphate); **CAT**, (catalase); **DAG**, (diacylglycerol); **EDTA**, (Ethylenediaminetetraacetic acid); **ETC**, (electron transport chain); **GPX**, (glutathione peroxidase); **GST,**, (glutathione S-transferase); **HSP**, (heat shock protein); **IPM**, (integrated pest management); **LC-MS/MS**, (Liquid Chromatography with tandem mass spectrometry); **NBT**, (nitrotetrazolium blue chloride); **NL**, (nonlinear); **PC**, (principal component); **PCA**, (Principal Component Analysis); ***p*NPP**, (4-nitrophenyl palmitate); ***p*NPPC**, (*p*-nitrophenylphosphorylcholine); **PR1**, (serine endoprotease); **PR2**, (trypsin-like acid protease); **PRX**, (peroxiredoxin); **PVP**, (polyvinylpyrrolidone); **ROS**, (reactive oxygen species); **SB**, (resuspension buffer); **SD**, (standard deviation); **SOD**, (superoxide dismutase); **TAG**, (triacylglycerol); **TCA**, (tricarboxylic acid cycle); **U**, (activity unit); **WSC**, (cell wall integrity and stress response components).

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1. Introduction

Advances in the agrochemical industry enable effective protection of crops against arthropods. Several synthetic insecticides are found on the market, pyrethroids being one of the most prominent. Pyrethroids are pyrethrin analogs that affect the nervous system of insects by disrupting the activity of chloride and sodium channels (Bhatt et al., 2019). They are applied in agricultural, forestry, and urban areas and show higher efficiency and lower toxicity compared with other insecticides (Tang et al., 2018).

Although until recently pyrethroids were considered less toxic to humans than other insecticides, it is now established that human exposure to these substances can lead to kidney and liver damage and disorders of the immune, endocrine, and nervous systems (Khalil et al., 2022). Pyrethroids have a long half-life in the soil, and their residues persist in the environment due to their lipophilic and hydrophobic properties. They accumulate in soils or enter rivers and sediments through rain scouring or other transport mechanisms (Tang et al., 2018; Abbas et al., 2022). Pyrethroids can be attached to the soil matrix, but no research has been conducted on whether soil microorganisms can also participate in this process by accumulating these toxic components in cells.

Entomopathogenic fungi are soil-dwelling fungi which are pests pathogens. Besides controlling insect populations, they perform numerous functions in the ecosystem, e.g., acting as plant endophytes (Behie and Bidochka, 2014) and participating in the removal of hazardous contaminants (Nowak et al., 2019). Commercial preparations based on entomopathogenic fungi seem to be an interesting and, most importantly, safe alternative to synthetic insecticides, the most popular being biopreparations based on fungi of the genera *Beauveria* and *Metarhizium* (Gao et al., 2020). It is worth mentioning that *Beauveria* is the most popular and important (both economically and environmentally) fungal pathogen of arthropods (Mayerhofer et al., 2015).

It is possible that soil-dwelling entomopathogenic fungi and pyrethroid pesticides can be in constant contact. Pyrethroids accumulate in soils even over long periods of time after application (Cycoń and Piotrowska-Seget, 2016). Entomopathogens are introduced into the environment (interchangeably with insecticides) during integrated pest management (IPM) programs and they can survive in the soil for many years, maintaining their activity (Mayerhofer et al., 2015).

Determining the effect of pyrethroids on entomopathogenic fungi is important, particularly at the cellular level because previous research explains only how pyrethroids affect growth parameters or individual enzymes and focuses on specific insecticides (Sun et al., 2011; Forlani et al., 2014; Oliveira et al., 2016).

Due to research gaps, the objectives of the current study are: (1) to determine the possibility of *B. bassiana* mycelium to accumulate pyrethroids (2) to evaluate the effects of accumulated insecticides on the activity of extracellular and intracellular enzymes involved in the infection process (3) to estimate the effect of accumulated pyrethroids on basic metabolism with proteomic background (4) to determine the effect of accumulated insecticides on oxidative stress. These findings are important because the accumulation of toxic compounds can affect the functioning of entomopathogenic fungi in the natural environment and also cause the deposition of these substances in fungal cells in environment, which could contribute to environmental pollution.

2. Materials and methods

2.1. Strain and growth conditions

In this study, the entomopathogenic fungus strain *Beauveria bassiana* ARSEF 2860 obtained from the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures, USA, was used. To inoculate 20 mL of Sabouraud dextrose broth medium, conidia harvested from 14-day cultures on Sabouraud dextrose agar slants were used. The precultures were incubated on a rotary shaker (120 rpm) for 48 h at 28 °C and transferred to Sabouraud dextrose broth medium (in a ratio of 1:9) supplemented with λ-cyhalothrin or α-cypermethrin or deltamethrin (Sigma-Aldrich, Germany) at final concentrations of 5 mg L^{-1} (stock solution 10 mg mL^{-1} in acetonitrile). The concentration of pyrethroids used in the study was selected based on the working concentrations recommended by the manufacturers of commercial preparations, which are: for λ-cyhalothrin up to 33 mg L^{-1} (data for commercial preparation Karate); for α-cypermethrin in the range of 41.5–150 mg L^{-1} (data for commercial preparation Cyperkill Max), for deltamethrin up to 49.95 mg L^{-1} (data for commercial preparation Deltam). The selected concentration, depending on the substance, is 6–30 times lower than the recommended working concentration. Using this concentration allowed us to demonstrate that *Beauveria* accumulates pyrethroids very efficiently. The samples were incubated at 28 °C and withdrawn for analysis at 48, 120, and 168 h.

2.2. Extraction and quantification of pyrethroids

The pyrethroid content in the biomass and the culture medium of *B. bassiana* was examined as described in our previous work (Litwin et al., 2021). In brief, 20 mL of the culture was filtered and the biomass was homogenized on an ultrasonic homogenizer. The culture medium and the homogenized biomass were extracted thrice with hexane. The quantity of pyrethroids was determined using a GC-MS system (Agilent) equipped with an HP 5 MS column (30 m × 250 μm×0.25 μm) and a 5975 C mass detector (Agilent).

2.3. Proteomic analyses

2.3.1. Protein extraction

The cultures were filtered using a filter unit of 0.45 μm pore size (Thermo Scientific). In order to isolate extracellular proteins, the culture medium was transferred to Falcon tubes, mixed well with cooled acetone in a ratio of 1:4, incubated overnight at − 20 °C, and centrifuged at 7000 ×*g* for 20 min at 4 °C. The obtained sediment was homogenized in a ball mill and centrifuged at 13.000 ×*g* for 5 min at 4 °C. The supernatant was then discarded, and the purified protein sediment was dried under reduced pressure and dissolved in SB buffer (7 M urea, 2 M thiourea, 4% CHAPS, 0.01 M DTT). The total protein concentration was determined using the Bradford method.

In order to isolate intracellular proteins, the method described by Mironenka et al. (2021) was used. In brief, 100 mg of the lyophilized biomass was cooled in liquid nitrogen and then homogenized in a ball mill. The obtained biomass powder was dissolved in SB buffer with protease inhibitors and centrifuged twice at 13.000 ×*g* for 15 min at 4 °C. The supernatant was transferred to new low-bind Eppendorf tubes and precipitated with low-cold acetone. The samples were vortexed and cooled for at least 60 min at − 20 °C. After centrifugation, the supernatant was discarded, and the precipitate was purified thrice with cooled acetone. Subsequently, acetone was removed from the samples under reduced pressure. The dried precipitate was dissolved in SB buffer (approximately 500 μL, depending on the quantity of the precipitate), and the total protein concentration was determined using the Bradford method.

2.3.2. 1-D SDS–PAGE

Extracellular proteins were separated using the SDS–PAGE method with a molecular mass marker of 6500 to 200.000 (Sigma Aldrich). The experiments were carried out in triplicate, and semiquantification was carried out using the GelAnalyzer 19.1 software (www.gelanalyzer.com; by Istvan Lazar Jr., Ph.D. and Istvan Lazar Sr., Ph.D., CSc).

2.3.3. 2-D SDS–PAGE

As described in a previous study, intracellular proteins were separated using 2-D SDS–PAGE electrophoresis (Soboń et al., 2019). In brief,

200 μg of protein was passively rehydrated on 11-cm IPG strips of pH 3–10 NL (nonlinear) (Bio-Rad, Germany). Isoelectric focusing was carried out on a Protean i12 (Bio-Rad, Germany) as follows: holding at 50 V for 4 h, then setting a linear gradient to 6000 V over 5 h, and holding for 120.000 Vh. After isoelectric focusing, the samples were incubated for 15 min in 1% DTT in equilibration buffer (50 mM Tris–HCl, 6 M urea, 30% (v/v) glycerol, and 2% SDS) and then for 15 min in 2% IAA in equilibration buffer. Electrophoresis was carried out on 12% SDS gels using a PROTEAN XL CELL device (Bio-Rad, Germany). As a gel calibrator, a molecular mass marker (6500–200.000 Da) (Sigma Aldrich) was used. The resulting gels were stained with Coomassie blue. The experiment was repeated thrice. The IMAGE MASTER 2D PLATINUM 7 software (GE Healthcare, Germany) was used to identify the differences. Spots that differed in intensity were excised from the gels, digested, and used for further analyses.

2.3.4. Protein identification

Spots excised from 1-D and 2-D gels were transferred to Eppendorf tubes and digested following the procedure of Soboń et al. (2019). The peptide sequences were read using matrix-assisted laser desorption ionization–time of flight (MALDI TOF/TOF Sciex 5800 TOF/TOF system, USA) following the method proposed by Mironenka et al. (2021). Proteins were identified using Protein Pilot v4.5 (Sciex) with the Mascot v2.4 search engine. The MS data were analyzed using the NCBI database with the taxonomy filter for *Beauveria*. All searches were evaluated based on the significant scores obtained from Mascot.

The protein score was set to > 95%, and a significance threshold of $p < 0.05$ was used.

2.4. Extracellular enzyme activity measurements

Of each culture, 1 mL was transferred to an Eppendorf tube and centrifuged at 7000 ×*g* for 5 min at 4 °C. The supernatant was collected, and the activity of extracellular enzymes was measured according to the following methods.

2.4.1. Lipase activity measurement

The lipolytic activity was determined following the method of Orlando Beys Silva et al. (2005). In brief, 20 μL of the culture supernatant was transferred to a 96-well plate and 80 μL reagent containing 0.03% *p*NPP (4-nitrophenyl palmitate) in 50 mM Tris–HCl buffer (pH 8.0), followed by the addition of 0.4% Triton X-100% and 0.1% Arabic gum. The samples were then incubated for 1 h at 37 °C. After incubation, using a FLUOstar Omega microplate reader (BMG Labtech, Germany), the absorbance of the samples was measured at a wavelength of 410 nm. The quantity of *p*-nitrophenol was calculated based on the standard curve prepared in the 1–100 μMol range (Bilen and Turan, 2022). The results were expressed as a unit amount of the enzyme which releases 1 μMol *p*-nitrophenol per minute.

2.4.2. Phospholipase C extracellular activity measurement

The extracellular phospholipase C activity was measured in the culture supernatant. Of the sample, 10 μL was mixed with 90 μL of a 20 mM solution of *p*NPPC (*p*-nitrophenylphosphorylcholine) in 50 mM Tris–HCl buffer (pH 8.0) in a microplate well. As a standard, a solution of *p*-nitrophenol in the concentration range of 0–100 μMol was used. The absorbance of the samples was measured at 410 nm before and after incubation for 1 h at 37 °C. The results obtained were evaluated following the method described by Zhao et al. (2020). The slope of the linear regression of the standard curve and the slope of the linear regression of the kinetic curve of the samples were used to calculate the enzymatic activity of the samples, which is expressed in μMol min^{-1}.

2.4.3. Extracellular protease activity measurement

2.4.3.1. Total proteolytic activity measurement. The proteolytic activity was measured following the procedure used by Perinotto et al. (2014) with 2% azocasein as the substrate. Results were expressed as the percentage of proteolytic activity of the samples in relation to the biotic control.

2.4.3.2. PR1 and PR2 activity measurement. To measure the activity of serine endoprotease (PR1) and trypsin-like acid protease (PR2), the method proposed by Dhar and Kaur (2010) using 1 mM of N-succiny-l-Ala-Ala-Pro-Phe-*p*-nitroanilide and benzoyl-Phe-Val-Arg-*p*-nitroanilide hydrochloride was used, respectively. The enzymatic activity of the samples was expressed as the amount of enzyme that liberates 1 μM *p*-nitroaniline per minute.

2.4.4. Chitinase activity measurement

The chitinolytic activity was determined following the method described by Agrawal et al. (Agrawal and Kotasthane, 2012) with minor modifications. Of the supernatant, 30 μL was transferred to a new Eppendorf tube with 70 μL of one of the substrates: *p*-nitrophenyl β-D-N, N,N-triacetylchitotriose, *p*-nitrophenyl N,N-diacetyl-β-D-chitobioside, and *p*-nitrophenyl N-acetyl-β-D-glucosaminide (for the determination of endochitinase triacetylchitotriosidase, chitobiosidase, and glucosaminidase, respectively). The assays were performed for 45 min at 37 °C in a 96-well microplate at pH 4.8. A sodium carbonate solution was used to stop the reaction, which resulted in the yellow color of the samples. The quantity of *p*-nitrophenol liberated was measured at a wavelength of 405 nm. The results were expressed as U/mL × 10^{-3}, whereas the activity unit (U) was defined as the quantity of the enzyme that releases 1 mmol of *p*-nitrophenol per minute.

2.4.5. Laccase activity measurement

The laccase activity was measured according to the method proposed by Góralczyk-Binkowsk et al. (2020) with 10 mM ABTS as the substrate. One unit of laccase activity (U) was defined as the quantity of enzyme that oxidizes 1 μM ABTS per minute.

2.5. Intracellular enzyme activity measurements

2.5.1. Activity of antioxidant enzymes

To evaluate the catalase (CAT) and superoxide dismutase (SOD) activities, 500 mg of wet biomass was withdrawn and homogenized in 0.05 M sodium phosphate buffer, pH 7, with 1 mM EDTA and 1% PVP. The samples were centrifuged at 15.000 ×*g* for 10 min at 4 °C, and the supernatant was transferred to new Eppendorf tubes. For CAT activity evaluation, H_2O_2 degradation was measured at 240 nm for 4 min. To determine the SOD activity, inhibition of nitrotetrazolium blue chloride (NBT) reduction at 540 nm was measured (Nykiel-Szymańska et al., 2019).

2.5.2. Phospholipase C intracellular activity

The intracellular phospholipase C activity was measured in the supernatant after biomass homogenization following the same procedure as described for extracellular phospholipase C activity measurement (Section 2.4.2).

2.6. Quantification of reactive oxygen species

The intracellular level of reactive oxygen species (ROS)—hydrogen peroxide, superoxide anion, and nitric oxide—was determined as previously described (Litwin et al., 2021) using 1 mg mL^{-1} DAB solution, 0.1% NBT, and 25 mM H_2DCFDA, respectively. The ROS content in *B. bassiana* cells was determined under an LSM510 Meta confocal laser scanning microscope (Zeiss). The results were presented as the

percentage of stained area compared with the total hyphal area.

2.7. Quantification of neutral lipids

The acylglycerol content was determined following the method of Stolarek et al. (2019) with some modifications. In brief, 100 mg of wet biomass with 1 mL of a chloroform–methanol mixture (2:1, v/v) was extracted twice using a ball mill and centrifuged at 5000 ×*g* for 5 min. Then, 200 μL of water was added to the supernatant, vortexed, and centrifuged. The bottom layer was collected, and after evaporation, the neutral lipids were dissolved in 1 mL of methanol. The content of neutral lipids was determined using LC-MS/MS. To detect acylglycerol, ammonium adducts of multiple reaction monitoring scans including parent–daughter pairs were used. Chromatographic separation was carried out on a C18 column heated to 40 °C, and detection was performed using single-ion monitoring and the enhanced product ion method. As mobile phases, water and a mixture of acetonitrile–isopropyl alcohol (5:2) containing 5 mM ammonium formate and 0.1% formic acid were used.

2.8. Statistical analyses

All samples were prepared in triplicate. The experiments were repeated twice, and the standard deviation (SD) was determined. The normality of the data distribution was assessed using the Shapiro–Wilk test. For normally distributed results, data were analyzed using one-way analysis of variance, and means were compared using Tukey's post hoc test. However, for results that were not normally distributed, the nonparametric Kruskal–Wallis test was used, and means were compared on the basis of the *p*-values for multiple comparisons. To evaluate the ability of *B. bassiana* to accumulate pyrethroids (2.2. Extraction and quantification of pyrethroids), for results that were not normally distributed, a nonparametric Mann–Whitney U test was used (suitable for two independent sample comparisons). A *p*-value of < 0.05 was considered statistically significant. Statistical analyses were carried out using the STATISTICA version 13.3 software (StatSoft). For the proteomic study, the proteins identified using Mascot with scores greater than 61 (5% confidence threshold) were considered statistically significant.

3. Results and discussion

3.1. Accumulation of pyrethroids on B. bassiana biomass

Pyrethroids added at concentrations (5 mg L^{-1}) to the culture medium accumulated in the mycelium of the entomopathogenic fungus *B. bassiana* just after 2 days of incubation (Table 1). The quantity of insecticides detected in *B. bassiana* cells was significantly higher than in the postculture medium with statistically significant differences after 2 and 7 days of culture for λ-cyhalothrin ($p = 0.005075$; $p = 0.005075$), α-cypermethrin ($p = 0.005075$; $p = 0.005075$) and deltamethrin ($p = 0.000187$; $p = 0.005075$) (Table 1).

Table 1
Pyrethroids accumulation in *B. bassiana* mycelium relative to the amount of pyrethroids supplied, accounting for extraction efficiency of pyrethroids from culture medium and culture medium with autoclaved mycelium. The initial pyrethroids concentration was 5 mg L^{-1}(1).

		Pyrethroids recovery [%]		
		λ -cyhalothrin	α -cypermethrin	Deltamethrin
Time (days)				
2	Abiotic control	94.00 ± 9.67	93.42 ± 9.18	88.77 ± 17.63
	Mycelium	94.95 ± 9.35**	90.51 ± 7.55**	86.40 ± 2.86**
	Post-culture - medium	0.53 ± 0.45	1.01 ± 0.47	0.53 ± 0.09
7	Abiotic control	86.60 ± 10.87	75.73 ± 10.61	80.04 ± 3.24
	Mycelium	86.26 ± 7.07**	59.38 ± 4.73**	75.30 ± 9.01**
	Post-culture - medium	0.06 ± 0.02	0.61 ± 0.14	0.02 ± 0.00

Data are expressed as means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice; n = 6.
Normally distributed data were analyzed using one-way analysis of variance, and means were compared using Tukey's post hoc test. Nonnormally distributed data were analyzed using the nonparametric Mann–Whitney U test. Statistically significant differences between the content of pyrethroids accumulated in the mycelium and the content of pyrethroids remaining in the post-culture medium were determined. Significance: **—$p < 0.01$, * —$p < 0.05$.
(1) Percent recovery was calculated relative to the amount of pyrethroids supplied, accounting for extraction efficiency of pyrethroids from culture medium and culture medium with autoclaved mycelium.

3.2. Influence of pyrethroid accumulation on the B. bassiana extracellular proteins

3.2.1. Effect of pyrethroid accumulation on B. bassiana lytic enzymes activity

3.2.1.1. Lipase activity. The lipase activity increased in all samples evaluated throughout cultivation. Pyrethroids accumulated in the *B. bassiana* biomass at 48 h (Table 1, Section 3.1) did not affect the lipolytic activity of this fungus. Similar results were obtained at 120 and 168 h, except for α-cypermethrin in 168-h cultures, which significantly increased the lipolytic activity ($p = 0.008111$) of *B. bassiana* (Table 2).

In proteomic studies, 66 kDa phospholipase C (accession: KAF1734622.1, score: 123) and 66 kDa phosphoesterase (accession: OAA40432.1, score: 120) were also detected in the postculture medium of *B. bassiana*. The pyrethroid accumulation did not affect the extracellular phospholipase C activity at 48 h of incubation only (Table 2). Accumulated λ-cyhalothrin increased the activity of this enzyme in the 120-h and 168-h cultures ($p = 0.003866$, $p = 0.000085$, respectively). The α-cypermethrin accumulation also caused an increase in the enzyme activity at 168 h ($p = 0.02984$).

3.2.1.2. Protease activity. Analyses of the extracellular proteome revealed the presence of various proteases in 48-h cultures of *B. bassiana*. The presence of 45 kDa tripeptidyl peptidase Sed1 (accession: KGQ06546.1, score: 123), 68 kDa alkaline serine protease (accession: EJP62249.1, score: 209), 51 kDa metalloprotease (accession: PMB63986.1, score: 118), 51 kDa trypsin-like serine protease (accession: PQK13155.1, score: 105), and 69 kDa peptidases belonging to the S8/S53 family (accession: OAA35631.1, score: 107), and 51 kDa S1 and S6 families (accession: OAA35492.1, score: 116) was confirmed, among others.

The accumulation of λ-cyhalothrin and α-cypermethrin resulted in a statistically significant increase in the total proteolytic activity at 48 h ($p = 0.002232$; $p = 0.000871$, respectively), 120 h ($p = 0.001270$; $p = 0.005699$, respectively), and 168 h ($p = 0.006545$; $p = 0.001964$, respectively), whereas deltamethrin showed no influence (Table 2). As revealed by extracellular proteome analyses, *B. bassiana* secretes many proteases, the most important being the PR1 and PR2, both of which play a crucial role in the insect infection process (Mondal et al., 2016; Gao et al., 2020).

Measurements of the PR1 and PR2 activities showed that λ-cyhalothrin ($p = 0.001739$) and α-cypermethrin ($p = 0.000000326$) caused a statistically significant decrease in the PR1 activity at 48 h, whereas the PR2 activity was reduced in the presence of α-cypermethrin ($p = 0.013407$). In 120-h cultures, all the tested substances caused a decrease in the PR1 activity ($p = 0.000000326$ for λ-cyhalothrin; $p = 0.040737$ for α-cypermethrin; $p = 0.038074$ for deltamethrin), and λ-cyhalothrin ($p = 0.000062$) and deltamethrin ($p = 0.019732$) also reduced the PR2

Table 2
Extracellular enzyme activity of *B. bassiana* with accumulated λ-cyhalothrin, α-cypermethrin, and deltamethrin.

			Pyrethroids (5 mg L^{-1})		
	Time [h]	Biotic control	λ-cyhalothrin	α-cypermethrin	Deltamethrin
Lipase activity[μMol min^{-1}]	48	0.42 ± 0.017	0.41 ± 0.017	0.41 ± 0.057	0.43 ± 0.044
	120	0.36 ± 0.025	0.47 ± 0.060	0.45 ± 0.043	0.42 ± 0.007
	168	0.66 ± 0.078	0.75 ± 0.071	0.82 ± 0.058**	0.69 ± 0.042
Phospholipase C extracellular activity [μMol min^{-1}]	48	0.079 ± 0.008	0.081 ± 0.010	0.089 ± 0.016	0.093 ± 0.009
	120	0.033 ± 0.016	0.095 ± 0.019**	0.081 ± 0.015	0.062 ± 0.013
	168	0.043 ± 0.012	0.585 ± 0.093**	0.146 ± 0.011*	0.104 ± 0.012
Protease activity [%]	48	100.00 ± 0.97	123.79 ± 10.35**	123.97 ± 3.60**	101.77 ± 1.47
	120	100.00 ± 5.96	110.71 ± 6.81**	109.22 ± 3.12**	104.15 ± 5.15
	168	100.00 ± 5.15	111.88 ± 1.92**	113.63 ± 4.66**	106.35 ± 3.59
PR1 activity [U]	48	53.12 ± 2.36	39.24 ± 1.73**	20.70 ± 0.65**	45.22 ± 1.61
	120	27.09 ± 0.94	20.57 ± 0.56**	23.98 ± 0.46*	23.96 ± 0.77*
	168	37.16 ± 0.95	28.91 ± 1.11	39.53 ± 0.95	54.69 ± 0.58**
PR2 activity [U]	48	36.30 ± 1.68	32.65 ± 1.77	26.85 ± 0.60*	33.27 ± 0.28
	120	35.54 ± 0.69	24.46 ± 0.11**	30.80 ± 0.11	30.29 ± 0.11*
	168	29.77 ± 0.27	20.40 ± 0.31	32.65 ± 0.16	34.36 ± 0.27*
Triacetylchitotrioside activity [U mL^{-1} × 10^{-3}]	48	0.23 ± 0.011	0.31 ± 0.011**	0.28 ± 0.015	0.26 ± 0.015
	120	0.27 ± 0.008	0.27 ± 0.006	0.29 ± 0.025	0.25 ± 0.013
	168	0.25 ± 0.034	0.20 ± 0.023**	0.21 ± 0.007**	0.20 ± 0.027**
Chitobiosidase activity[U mL^{-1} × 10^{-3}]	48	0.12 ± 0.015	0.29 ± 0.012**	0.22 ± 0.045	0.33 ± 0.111**
	120	0.60 ± 0.097	0.47 ± 0.053	0.45 ± 0.041**	0.51 ± 0.050
	168	0.48 ± 0.050	0.44 ± 0.041	0.49 ± 0.041	0.54 ± 0.032
N-acetyl-glucosaminidase activity[U mL^{-1} × 10^{-3}]	48	7.10 ± 0.077	7.38 ± 0.123**	6.58 ± 0.052**	7.16 ± 0.095
	120	12.65 ± 0.828	11.04 ± 0.128**	11.06 ± 0.377**	11.75 ± 0.442
	168	10.98 ± 0.319	10.54 ± 0.566	10.46 ± 0.340	11.08 ± 0.435
Laccase activity [U L^{-1} min^{-1}]	48	1.29 ± 0.005	1.43 ± 0.009*	1.78 ± 0.017**	1.37 ± 0.010
	120	1.01 ± 0.077	1.06 ± 0.082	1.32 ± 0.056*	1.38 ± 0.068**
	168	1.45 ± 0.058	1.35 ± 0.042	1.36 ± 0.119	1.52 ± 0.091

Data are expressed as means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice; .n = 6.
Results with a normal distribution were tested using one-way analysis of variance, whereas nonparametric Kruskal–Wallis test was used to analyze the results that were not normally distributed; significance: **—$p < 0.01$, * —$p < 0.05$.

activity. Interestingly, an increase in the PR1 and PR2 activities was observed only in the presence of deltamethrin after 168 h of incubation ($p = 0.009076$; $p = 0.019732$, respectively), which could be because deltamethrin accumulates the least in biomass at 168 h (Table 1, Section 3.1).

3.2.1.3. Chitinase activity. Proteomic analyses revealed the presence of chitinase in the tested samples. The presence of 34 kDa endo-beta-N-acetylglucosaminidase (accession: PMB64956.1) was found after 48 h in the biotic control and in the samples with α-cypermethrin (score: 223), and after 168 h in all the tested samples (score: 337). Measurements of the enzymatic activity of endochitinase (triacetylchitotriosidase) and two exochitinases (chitobiosidase and N-acetylglucosaminidase) revealed that N-acetylglucosaminidase is the dominant type of chitinase in *B. bassiana* (Table 2).

It was observed that the accumulation of λ-cyhalothrin caused an increase in the activity of all tested chitinases in the 48-h cultures ($p = 0.000244$; $p = 0.009818$; $p = 0.002187$, respectively). A similar phenomenon was observed for chitobiosidase in the samples with accumulated deltamethrin ($p = 0.000745$). On the contrary, the accumulation of α-cypermethrin caused a decrease in the N-acetylglucosaminidase activity in the 48-h ($p = 0.016011$) and 120-h cultures ($p = 0.000857$). At 168 h, endochitinase was inhibited in all the tested samples (λ-cyhalothrin ($p = 0.000186$), α-cypermethrin ($p = 0.003008$), and deltamethrin ($p = 0.000253$), whereas the tested exochitinases activity did not change in comparison with the control (Table 2).

3.2.2. Laccase activity

In the proteomic analyses of this study, the presence of 67 kDa laccase (accession: EJP62796.1, score: 233) was confirmed in all the samples tested. The accumulation of λ-cyhalothrin and α-cypermethrin significantly increased the laccase activity ($p = 0.019732$; $p = 0.0000632$, respectively) at 48 h of incubation. For α-cypermethrin, this effect lasted up to 120 h, when an increase in the laccase activity was also observed for samples with deltamethrin ($p = 0.041259$; $p = 0.004727$, respectively) (Table 2).

Insert Table 2.

3.2.3. Other extracellular proteins

Based on the analyses of the extracellular proteome of *B. bassiana,* in addition to the lytic enzymes and laccase described earlier, the presence of other proteins involved in various processes was also demonstrated. Some proteins were found only in the samples with pyrethroids. Antigenic thaumatin-like protein (16 kDa, accession: KAF1732013.1, score: 65) was detected in 48-h cultures with the addition of deltamethrin. This protein exerts antifungal activity by disrupting the synthesis of the fungal cell wall, may protect entomopathogenic fungi against other pathogenic fungi, and may prevent competition among fungal species during the infection process (Liu et al., 2010).

Proteins related to the development of hyphae and the cell wall were also detected, e.g., 56 kDa glucanosyltransferase (accession: PQK09149.1, score: 94) in the samples with accumulated λ-cyhalothrin and α-cypermethrin at 48 h of incubation and 48 kDa glucan endo-1,6-beta-glucosidase B (accession: KGQ06570.1, score: 380), 48 kDa beta-1,6-glucanase (accession: EJP62259.1, score: 353), and 48 kDa glucan 1,3-beta-glucosidase (accession: OAA35640.1, score: 315) in the samples with accumulated α-cypermethrin at 48 h and in the samples with λ-cyhalothrin and α-cypermethrin at 168 h. The presence of these proteins may imply that the accumulation of the pyrethroids damages the cell wall, which is then regenerated, and the results revealed that λ-cyhalothrin exerted the strongest effect.

Divergent proteases such as metalloprotease StcE, peptidase S1 and S6, chymotrypsin/Hap, and hypothetical protein BB8028_0004g00860—Trypsin-like serine protease were detected in 48-h samples with accumulated α-cypermethrin and deltamethrin. These observations are consistent with our previous results with respect to the total proteolytic activity, which was found to increase in the presence of α-cypermethrin.

3.3. Influence of accumulated pyrethroids on B. bassiana intracellular proteins

To reveal the most noticeable changes in *B. bassiana* cells during the accumulation of pyrethroids, intracellular proteins were identified using MALDI TOF/TOF analyses. The obtained results are presented in Figs. 1–2 and Supplementary Tables S1–S2, including the locations of spots in the gel images (Supplementary Figs. S1–S2).

The results of the identified proteins described in the study constitute the average of three replicates of gels and meet the statistical significance criterion $p < 0.05$.

3.3.1. Proteins associated with pyrethroid accumulation

In the biomass with accumulated α-cypermethrin, overexpression of cyanate hydratase (spots 1 and 71), cyanide hydratase (spots 72–78) and flavohemoprotein (spots 79–80) was observed (Figs. 1–2, Supplementary Figs. S1–S2 and Supplementary Tables S1–S2).

During incubation, overproduction of proteins involved in the organization of the actin cytoskeleton—cofilin (spots 4–5 and 84–86), actin lateral binding protein (spot 81), and F-actin capping protein subunit (82)—was observed in the biomass with accumulated α-cypermethrin.

In addition, deltamethrin induced alcohol dehydrogenase in the 48-h samples (spot 66) but inhibited the production of this enzyme in 168-h samples (spots 142–146).

3.3.2. Enzymes of metabolic pathways

The expression of the proteins involved in glycolysis (spots 6–9), tricarboxylic acid cycle (TCA, spots 10–11), the electron transport chain (ETC, spots 13–16 and 18), and the pentose phosphate cycle (spot 12), which are crucial to the development and growth of the fungus, was reduced in 48-h biomass with accumulated λ-cyhalothrin (Fig. 1 and Supplementary Table S1). Interestingly, an increase in the expression after 168 h of culture was observed (spots 100, 102–104; Supplementary Table S2) only for the enzymes involved in ETC.

Protein expression was not significantly altered by accumulated α-cypermethrin after 48 h of incubation, but enzymes involved in the metabolism of azo compounds (spots 1, 71–78, and 80; Supplementary Tables S1–S2), the formation of cell walls and hyphae, and the reduction of peroxides (spots 111 and 112, Supplementary Table S2) were overexpressed. Furthermore, a decrease in the quantity of cypermethrin in *B. bassiana* cells was probably related to an increase in the expression of enzymes involved in glycolysis (spots 87–89, 91–92, and 95–96), TCA cycle (spots 97–98), the pentose phosphate pathway (spot 99), and ETC (spots 100, 102, and 106) after 168 h (Supplementary Table S2).

The accumulation of deltamethrin in 48-h cultures influenced the metabolism of *B. bassiana* in a manner remarkably different from all other pyrethroids studied. Deltamethrin induced the expression of the basic metabolism of the tested fungus, which manifested in an increase in the expression of glycolysis enzymes (spots 6–9), the TCA cycle (spot 11), the pentose phosphate cycle (spot 12), and ETC (spots 13, 15, and 17–18). Other proteins involved in energy production were also overexpressed (ADP/ATP transporter on adenylate translocase and adenosine kinase, spots 19–20) (Supplementary Table S1).

The accumulation of pyrethroids also influenced chaperones and other proteins involved in protein metabolism. In λ-cyhalothrin and α-cypermethrin 48-h cultures, the expression of cyclophilin A and B (spots 31 and 32, Supplementary Table S1) was inhibited; also, in 168-h cultures, the expression of wos2 proteins (spot 120), cyclophilin A (spot 121), and peptidyl-prolyl cis-trans isomerase (spot 122) was reduced (Supplementary Table S2).

3.3.3. Accumulation of pyrethroids and oxidative stress

Antioxidant enzymes are involved in the protection of cells against the effect of oxidative stress. Proteomic studies showed the presence of different enzymes of oxidative stress in the *B. bassiana* proteome, both in the biotic controls and in the tested samples, as follows: CAT (spots 22 and 108–109), SOD (spots 21 and 107), glutathione S-transferase (GST, spots 23 and 110), glutathione peroxidase (GPX, spot 111), peroxiredoxin (PRX, spots 24 and 112), HSP 60 (spots 25 and 113), HSP (spots 116–117), HSP 70 (spots 26–28 and 114), HSP70-like protein (spots 29 and 115), HSP 30 (spot 118), and carbohydrate-binding WSC domain protein (spot 119) (Supplementary Figs. S1-S2 and Supplementary Tables S1-S2).

This study demonstrated that the accumulation of pyrethroids in the mycelium of *B. bassiana* (Table 1, Section 3.1) induced oxidative stress. Proteomic analyses showed changes in SOD expression in the presence of λ-cyhalothrin, α-cypermethrin, and deltamethrin in 168-h cultures. SOD activity measurements revealed an increase in this enzyme activity only in 168-h samples with deltamethrin (biotic control: 1.67 ± 0.20 U protein mg^{-1}; samples with deltamethrin: 2.75 ± 0.15 U protein mg^{-1}; $p = 0.037398$). Although pyrethroids have been shown to increase the CAT expression in the proteome, this increase was not confirmed during enzyme activity measurements in this study.

The influence of pyrethroids on the content of other antioxidant enzymes was also observed (Supplementary Figs. S1–S2 and

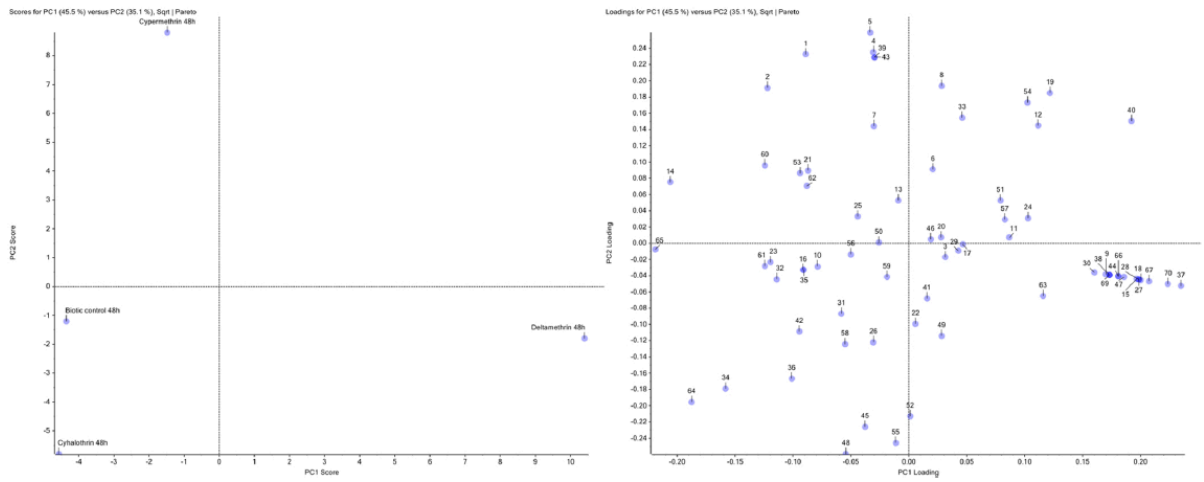

Fig. 1. PCA of the intracellular protein composition in biotic controls and samples with the addition of λ-cyhalothrin, α-cypermethrin, and deltamethrin after 48 h of culture. The numbers on the PC loading section represent individual spots (according to the numbering of spots in Supplementary Table S1).

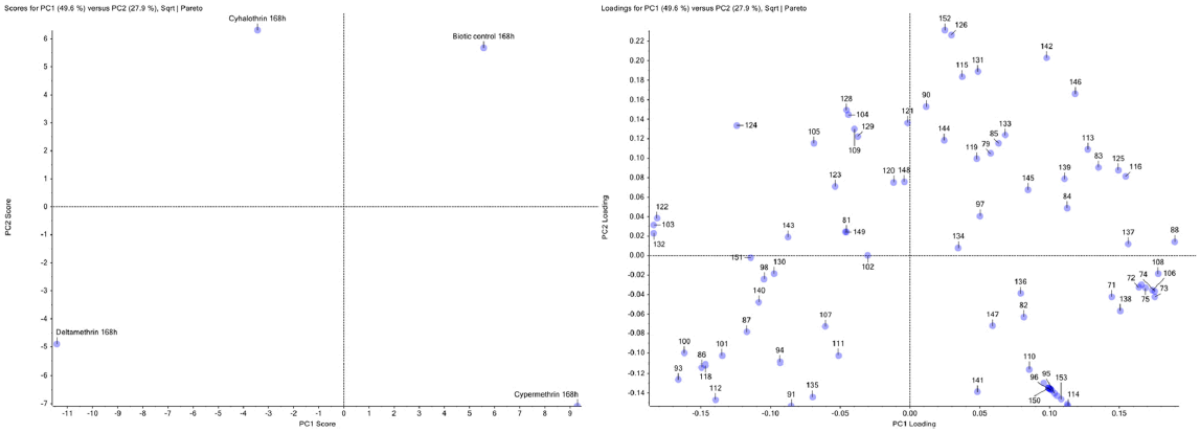

Fig. 2. PCA of the intracellular protein composition in biotic controls and samples with the addition of λ-cyhalothrin, α-cypermethrin, and deltamethrin after 168 h of culture. The numbers on the PC loading section represent individual spots (according to the numbering of spots in Supplementary Table S2).

Supplementary Tables S1–S2). It was observed that the presence of pyrethroids in 168-h cultures induced the production of GPX (spot 111), which belongs to the glutathione system and is responsible for the reduction of peroxides by converting two reduced glutathione molecules into glutathione disulfide (Huarte-Bonnet et al., 2015). In 48-h cultures, the addition of pyrethroids decreased the content of another enzyme of the glutathione system–GST (spots 23 and 110), whereas in 168-h cultures, the presence of this enzyme was demonstrated only in samples with the addition of α-cypermethrin. GSTs remove glutathione from some substrates and catalyze its coupling with hydrophobic compounds (containing an electrophilic nitrogen, carbon, or sulfur atom), which leads to the metabolism of toxic substances as well as peroxides (Morel et al., 2009; Huarte-Bonnet et al., 2015).

It was observed that deltamethrin in 48-h cultures increased the expression of PRXs (spot 24) and HSPs (spots 25–29). PRXs are considered one of the most important cellular defense systems against oxidative stress (Breitenbach et al., 2015) and are capable of reducing a wide spectrum of ROS (e.g., hydrogen peroxide, alkyl and lipid hydroperoxides, nitric oxide, peroxynitrite) (Rocha et al., 2018). HSPs are stress proteins (Xu et al., 2013), and also act as a marker of oxidative stress (Soboń et al., 2019). As reported by Xu et al. (2013), HSPs induce temporary changes in the metabolic activity of cells, which contributes to the protection of cells against further damage. Both PRXs and HSPs influence the infectivity of entomopathogenic fungi (Wang et al., 2020; Boysen et al., 2021). Furthermore, it was also observed that the addition of deltamethrin decreased the protein content of the carbohydrate-binding WSC domain protein, which is responsible for maintaining cell wall integrity and involved in the response to stress (Oide et al., 2019).

It was also observed that pyrethroids induce ROS production in *B. bassiana* hyphae (Table 3). An increase in the superoxide anion content was observed in the presence of α-cypermethrin and deltamethrin in 48-h cultures ($p = 0.036013$, $p = 0.008442$, respectively) and in the presence of λ-cyhalothrin and deltamethrin in 168-h cultures ($p = 0.001142$, $p = 0.012669$, respectively). An increase in the NO• content was also observed in the samples containing α-cypermethrin and deltamethrin, both in 48-h cultures ($p = 0.001067$, $p = 0.0000951$, respectively) and in 168-h cultures ($p = 0.000003$, $p = 0,\ 000025$, respectively).

Based on the results obtained, it can be concluded that deltamethrin causes the highest degree of oxidative stress in the tested fungi. Its presence induces the production of $O_2^{\bullet-}$ and NO•, and increases the SOD activity and the HSP content, which are considered stress markers.

In 168-h biotic controls, a high content of H_2O_2 was observed, while the enzyme responsible for its detoxification - CAT remained at a comparable level in both the control and the test samples. This could include activating a different defense mechanism for which proteins have been identified by proteomic studies (GST, spot 110 and GPX, spot 111 – Supplementary Table S2).

Table 3
Percentage of hyphae containing reactive oxygen species of *B. bassiana* with accumulated pyrethroids.

			Pyrethroids (5 mg L^{-1})		
		Biotic control	λ-cyhalothrin	α-cypermethrin	Deltamethrin
H_2O_2	48 h	nd	nd	nd	nd
	168 h	3.50 ± 1.32	1.14 ± 0.38*	1.43 ± 0.12	2.20 ± 0.44
$O_2^{\bullet-}$	48 h	0.60 ± 0.06	1.49 ± 0.13	4.26 ± 1.08 *	4.65 ± 0.84**
	168 h	1.10 ± 0.33	5.25 ± 0.89**	2.32 ± 0.70	3.90 ± 0.51 *
NO•	48 h	0.22 ± 0.03	0.39 ± 0.13	0.67 ± 0.15**	0.79 ± 0.21**
	168 h	0.04 ± 0.01	0.16 ± 0.03	0.25 ± 0.07**	0.23 ± 0.09**

Data are expressed as means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice; n = 6.
Results with a normal distribution were tested using one-way analysis of variance, whereas nonparametric Kruskal–Wallis test was used to analyze the results that were not normally distributed; significance: **—$p < 0.01$, * —$p < 0.05$.

3.3.4. *Effect of pyrethroids on intracellular phospholipase C activity in entomopathogenic fungi*

Intracellular phospholipases of microscopic fungi play several important roles both in cellular and physiological processes and in resistance to oxidative stress. They participate in the fungal cell signaling through their role in the Ca^{2+} signal transduction pathways and in phosphatidylinositol turnover (Barman et al., 2018). The role of phospholipase C in the Ca^{2+} signaling pathways confirms its importance for the proper functioning of organisms. Phospholipases also play an important role during fungi growth, hyphal development, and conidiation (Barman et al., 2018). They also affect the infection process by supporting the dissemination of the fungus in the insect's hemocell and avoiding the host's immune mechanisms (Djordjevic, 2010).

The influence of the accumulated pyrethroids on the intracellular phospholipase C activity depended on both the duration of incubation and the substance used. Pyrethroids caused a decrease in the

intracellular phospholipase C activity in 48-h cultures (1.33-fold by λ-cyhalothrin ($p = 0.000309$), 1.24-fold by α-cypermethrin ($p = 0.002517$), and 1.19-fold by deltamethrin ($p = 0.008399$)) and in 168-h cultures (1.15-fold by α-cypermethrin ($p = 0.001137$), 1.11-fold by deltamethrin ($p = 0.016011$)). Interestingly, the phospholipase C activity increased in the 120-h biomass with λ-cyhalothrin (1.20-fold, $p = 0.003478$) (Supplementary Table S3).

3.3.5. *Effect of accumulated pyrethroids on neutral lipids*

Acylglycerols play many important functions in entomopathogenic fungi and protect cells against oxidative stress (Fan et al., 2017). The total acylglycerol content decreased with the cultivation time in both the biotic controls and all the samples tested with the addition of pyrethroids (Table 4).

It was also observed that the accumulation of α-cypermethrin and deltamethrin increased the total TAG content: α-cypermethrin in 24-h and 120-h cultures ($p = 0.003631$; $p = 0.000267$, respectively) and deltamethrin in 48-h cultures ($p = 0.033010$). However, the accumulation of λ-cyhalothrin did not affect the total TAG content but decreased the total DAG content in 168-h cultures ($p = 0.000533$). The presence of α-cypermethrin and deltamethrin did not influence the total DAG content (Table 4).

An interesting finding is the fact that at 24 h of cultivation, the DAG content was higher than the TAG content, but at 48 h of cultivation, this dependence changed, and at later cultivation hours, the TAG content exceeded the DAG content (and this difference increased with the cultivation time).

It was observed that α-cypermethrin and deltamethrin increased the TAG/DAG ratio compared with the biotic control (α-cypermethrin at 24 ($p = 0.011506$), 120 ($p = 0.011506$), and 168 h of cultivation ($p = 0.003631$), and deltamethrin at 48 h of cultivation ($p = 0.017076$)) (Table 4). The results of the present study also showed that the accumulation of insecticides significantly influences the dependence between TAG and DAG on the *B. bassiana* biomass.

Using LC-MS/MS, 13 TAG species and 9 DAG species were identified in the mycelium of *B. bassiana* (Supplementary Table S4). The quantity of unsaturated acylglycerols was found to exceed that of the saturated acylglycerols. The primary molecular TAGs detected were 54:3, and 54:4, and DAGs were 32:0, 36:1, and 36:2. The accumulation of pyrethroids caused significant changes in the content of individual TAGs and DAGs (Supplementary Table S4). Accumulated α-cypermethrin had the highest impact on individual acylglycerols and caused a 1.7-fold increase in TAG 52:4 in 120-h cultures ($p = 0.000091$) and a 3- and 1.7-fold increase in TAG 54:6 in 24- and 120-h cultures ($p = 0.003631$, $p = 0.002679$, respectively). In addition, α-cypermethrin caused many changes in the TAG and DAG contents in 120- and 168-h cultures. Deltamethrin was also shown to cause an almost twofold increase in DAG 34:1 in 24-h cultures ($p = 0.033010$). The studied pyrethroids also caused a statistically significant decrease in the content of individual acylglycerols, but these differences were not as large as in the case of the increase in the TAG and DAG contents.

4. Discussion

Progress in the agrochemical industry enables effective protection of crops from arthropods, and pyrethroid insecticides are the most common and widely used in this area. They are the least poisonous and most effective insecticides available (Tang et al., 2018), yet they have a negative impact on human health, causing kidney and liver diseases and disrupting the functioning of the endocrine, nervous and immune systems (Khalil et al., 2022). Bioinsecticides based on entomopathogenic fungi seem to be a safe alternative to pyrethroids. Understanding the effects and consequences of exposing entomopathogenic fungi to pyrethroids seems to be very important, as they are in constant contact due to their interchangeable use in integrated pest management (IPM) programs. The research that has been done so far only consider the influence of pyrethroids on the growth or activity of individual enzymes, and the investigations cover just a small number of factors and are narrowly focused on certain pesticides (Forlani et al., 2014).

This paper reports the results of a wide range of mechanisms and functions of the entomopathogenic fungus *B. bassiana*, which is affected by three pyrethroid insecticides - λ-cyhalothrin, α-cypermethrin and deltamethrin. This study appears to be the first to find that pyrethroids accumulated in large quantities in the mycelium of *B. bassiana* had various impacts on extracellular enzymes necessary for pathogenesis, enzymes of the basic metabolic pathways, and enzymes responsible for the proper organization of the actin cytoskeleton and cell walls, as well as for the induction of oxidative stress.

The results presented in this study significantly expand the knowledge on the effects of pyrethroid insecticides on entomopathogenic fungi and the consequences of their direct contact. Herein, many previously unknown aspects of the influence of these substances' effects on entomopathogenic fungi is presented.

This study showed that λ-cyhalothrin, α-cypermethrin and deltamethrin (at concentrations 5 mg L^{-1}) accumulated in the mycelium of *B. bassiana* already after 2 days of incubation. These results complement our previous reports suggesting the possibility of the accumulation of pyrethroid and neonicotinoid insecticides in the mycelium of *B. bassiana* and *Metarhizium* sp. (Nowak et al., 2020; Litwin et al., 2021). In addition to our reports, the remaining available data show only the ability to accumulate these substances in plant and animal tissues (Tang et al., 2018). Since our results indicate that *B. bassiana* can accumulate pyrethroids in the mycelium, it is possible that these compounds affect fungal metabolism.

Table 4
Acylglycerols content [mg g^{-1} dry mass of lipids] and TAG/DAG ratio of *B. bassiana* with accumulated pyrethroids.

		Pyrethroids (5 mg L^{-1})			
		Biotic control	λ –cyhalothrin	α –cypermethrin	Deltamethrin
TAG	24 h	6.43 ± 0.47	6.98 ± 1.21	10.04 ± 0.34**	7.26 ± 0.66
	48 h	4.63 ± 0.93	5.15 ± 0.60	5.77 ± 0.77	6.38 ± 0.79*
	120 h	1.79 ± 0.06	2.14 ± 0.20	2.57 ± 0.20**	2.17 ± 0.22
	168 h	2.18 ± 0.26	2.18 ± 0.30	2.50 ± 0.18	2.20 ± 0.28
DAG	24 h	12.19 ± 4.24	12.41 ± 1.52	11.99 ± 1.46	11.57 ± 1.05
	48 h	4.19 ± 0.47	4.90 ± 0.39	4.41 ± 0.75	4.43 ± 0.43
	120 h	1.36 ± 0.24	1.33 ± 0.13	1.35 ± 0.01	1.44 ± 0.09
	168 h	1.04 ± 0.09	0.83 ± 0.02**	0.90 ± 0.07	0.93 ± 0.03
TAG/DAG	24 h	0.59 ± 0.22	0.56 ± 0.03	0.84 ± 0.07*	0.63 ± 0.02
	48 h	1.11 ± 0.20	1.05 ± 0.09	1.34 ± 0.26	1.44 ± 0.05*
	120 h	1.36 ± 0.30	1.61 ± 0.14	1.91 ± 0.16*	1.51 ± 0.06
	168 h	2.10 ± 0.29	2.63 ± 0.43	2.77 ± 0.07**	2.36 ± 0.24

Data are expressed as means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice; n = 6.
Results with a normal distribution were tested using one-way analysis of variance, whereas nonparametric Kruskal–Wallis test was used to analyze the results that were not normally distributed; significance: **—$p < 0.01$, * —$p < 0.05$.

The key enzymes involved in the infection process are lipases, proteases, and chitinases (Litwin et al., 2020). In the case of lipolytic enzymes, an increase in lipase activity was demonstrated in 168-h cultures with the addition of α-cypermethrin and an increase in the activity of extracellular phospholipase C in 120-h cultures with the addition of λ-cyhalothrin and 168-h cultures with the addition of λ-cyhalothrin and α-cypermethrin.

In our study, the presence of various proteases in the *B. bassiana* proteome was shown. Santi et al. (2010) discovered at least five protease isoforms in *Metarhizium anisopliae* cultures and concluded that this diverse set of proteases provides better adaptability for fungi to a wide variety of environmental conditions. Pyrethroid accumulation was found to decrease the activity of key virulence factors PR1 and PR2, while the overall proteolytic activity was increased. These results are in agreement with those of our proteomics studies, which revealed the presence of multiple proteases belonging to different families that are likely to enhance the overall proteolytic activity. Gao et al. (2020) showed that among the 11 Pr1 genes, only five participate in epidermis degradation.

Chitinases in entomopathogenic fungi, in addition to degrading the insect epidermis during the infectious phase, are also responsible for the remodeling of the cell walls during hyphae growth, connection, and branching (Mondal et al., 2016). Our proteomic studies revealed the presence of extracellular endo-beta-N-acetylglucosaminidase after 48 h in the biotic control and in the samples with α-cypermethrin, and after 168 h in all the tested samples. These results are in accordance with the measurements of the chitinolytic activity which showed that N-acetylglucosaminidase is the dominant type of chitinase in *B. bassiana*. The influence of accumulated pyrethroids on the chitinolytic activity is evident in a variety of ways. λ-Cyhalothrin seems to intensify this enzymes production, while α-cypermethrin decreases their activity. Deltamethrin appears to have only a slight influence on the chitinolytic activity of *B. bassiana*. The data suggests that potentially accumulated pyrethroids can interfere with the infection process as well as hyphae growth and remodeling (Mondal et al., 2016). An increase in the production of chitinases and N-acetyl-beta-glucosaminidase in entomopathogenic fungi *B. bassiana*, *Beauveria brongniartii,* and *Isaria fumosorosea* was also observed in cultures with toxic cobalt ions, as reported by Łopusiewicz et al. (2020).

Fungal laccases play an important role in many biological processes. *B. bassiana* has been reported to secrete laccase 2 (BbLac2) during the infection process, eliminating the ROS produced by the insect (Lu et al., 2021). In the proteomic analyses of this study, the presence of laccase was confirmed in all samples tested. Pyrethroids have also been shown to increase laccase activity. Although the occurrence of high levels of laccase activity in the presence of hazardous chemicals is a well-known phenomenon (Góralczyk-Binkowska et al., 2020), an increase in laccase secretion due to the accumulation of toxic compounds in fungi has not previously been observed. At the beginning of the accumulation of pyrethroids by *B. bassiana*, the induction of oxidative stress and the production of $O_2^{\bullet-}$ and NO• were also observed. Presumably, the increase in laccase activity could be a response to the pyrethroid accumulation process, as well as to ROS production. Laccase could also contribute to culture detoxification, as evidenced by a decrease in the content of λ-cyhalothrin, α-cypermethrin, and deltamethrin in the *B. bassiana* mycelium. A similar phenomenon was observed for bacterial laccase, which facilitated the detoxification of α-cypermethrin (Gangola et al., 2018).

For the first time, during our proteomic studies, we also identified proteins that were only present in *B. bassiana* cells with accumulated pyrethroids. Deltamethrin induces the synthesis of a set of extracellular proteins such as antigenic thaumatin-like protein, while λ-cyhalothrin and α-cypermethrin induces the synthesis of proteins associated with the development of hyphae and cell wall. Proteases: metalloprotease StcE, peptidase S1 and S6, chymotrypsin/Hap, and trypsin-like serine protease were detected in samples with accumulated α-cypermethrin and deltamethrin.

During these studies, intracellular proteins produced in *B. bassiana* cells with accumulated pyrethroids were also identified. α-Cypermethrin induced enzymes involved in the metabolism of azo compounds (cyanate hydratase, cyanide hydratase and flavohemoprotein). Cyanide hydratase converts hydrogen cyanide to formamide (Martínková et al., 2015). Due to the presence of a cyanide group in pyrethroid molecules, this enzyme can be involved in reducing its toxicity, as proposed in our previous work during alachlor biodegradation (Szewczyk et al., 2015). Additionally, flavohemoprotein, which reduces a variety of substrates in vitro and protects organisms against toxic nitrogen compounds during nitrosative stress, could play a protective role (Hausladen et al., 1998). Moreover, accumulated α-cypermethrin also induced overproduction of proteins involved in the organization of the actin cytoskeleton (cofilin, actin lateral binding protein and F-actin capping protein subunit). This result may indicate that α-cypermethrin does not affect the cell shape, supporting our earlier findings (Litwin et al., 2021). The accumulation of deltamethrin in 48 h cultures induced the production of alcohol dehydrogenase, which converts alcohols and the corresponding aldehydes and ketones and plays a role in the metabolism of xenobiotics (Łaniewska-Dunaj et al., 2013). It seems that the overproduction of this enzyme by *B. bassiana* is intended to protect cells against this harmful substance.

This study also demonstrated the effect of pyrethroids on the enzymes of the basic metabolic pathways - glycolysis, TCA, ETC, pentose phosphate cycle. It was found that λ-cyhalothrin in 48-h cultures causes a decrease in the content of these enzymes, while α-cypermethrin after 168-h cultures - an increase in the expression of enzymes involved in these processes. Deltamethrin was also shown to induce the expression of basic metabolism and increase the content of other enzymes involved in energy production. Despite the positive effect of deltamethrin on basic metabolism of *B. bassiana* in 48-h cultures, it also caused the highest degree of oxidative stress, the appearance of ROS, an increase in the content of enzymes responsible for the reduction of peroxides, and the presence of HSP. HSPs, in addition to having a protective function, also participate in the stress response, as described below. After 168 h of incubation, deltamethrin caused decrease in the expression of the enzymes of the basic metabolic pathways. There are no data in the literature on the effects of λ-cyhalothrin, α-cypermethrin and deltamethrin on the expression of the enzymes involved in the key metabolic pathways in *B. bassiana.*

Our studies also revealed that the accumulation of pyrethroids affected chaperones and other proteins involved in protein metabolism, resulting in a decrease in the expression of cyclophilin A and B, the wos2 proteins and the peptidyl-prolyl cis-trans isomerases. The wos2 protein plays an important role in protein folding and also binds to the chaperone, and HSP90 proteins, cyclophilins, and peptidyl-prolyl cis-trans isomerases are chaperones and are an important part of cell signaling (Wang and Heitman, 2005).

The influence of the accumulated pyrethroids on *B. bassiana* is varied. λ-cyhalothrin and α-cypermethrin in the initial hours of cultivation had a negative effect on the tested fungus, but after 168 h of incubation, the adverse effect of the investigated chemicals on the proteome also decreased. However, deltamethrin shows an entirely distinct effect that needs further investigation.

The accumulation of pyrethroids induces oxidative stress in *B. bassiana*. In 168-h cultures with deltamethrin, an increase in SOD activity and the presence of various oxidative stress enzymes were found in the proteome of *B. bassiana.* Additionally, pyrethroids induce ROS production in *B. bassiana* hyphae. The data from the present study are consistent with the observed increase in SOD and CAT activity on 4 day of cultivation in the presence of deltamethrin at a concentration of 250 μg cm^{-2} (Forlani et al., 2014). That effect is typical xenobiotics are accumulated by the cells of living organisms. Increased levels of antioxidant enzymes are commonly found to overcome the oxidative stress generated by toxic compounds (Turan, 2021, 2022; Rasool et al., 2022;

Tauqeer et al., 2022). Accumulated pyrethroids have also been shown to affect the activity of intracellular phospholipase C. In the 48-h and 168-h cultures, this enzyme activity decreased in biomass with accumulated insecticides, while λ-cyhalothrin in the 120-h cultures caused an increase. Intracellular phospholipase C influences, inter alia, the calcium ion signal transduction pathway. The observed decrease in intracellular phospholipase C activity may lead to decreased calcium ion signal transmission, which is supported by our earlier findings that the calcium content decreases in 48-h cultures with α-cypermethrin and deltamethrin at a concentration of 5 mg L^{-1} (Litwin et al., 2021).

In this study, it was shown that pyrethroids influenced total acylglycerol content. It was also shown that with the cultivation time the total content of acylglycerols decreased and the relationship between the amount of TAG and DAG changed.

At 24 h of cultivation, the DAG content was higher, but at 48 h of cultivation, the TAG content exceeded the DAG content. Similar results were obtained in *Umbelopsis isabellina* and 2,4-D studies by Bernat et al. (2018). α-Cypermethrin and deltamethrin have also been shown to increase the TAG/DAG ratio compared to that of the biotic control.

The observed increase in the total TAG content with a stable amount of DAG may be due to the decrease in the phospholipase C activity in *B. bassiana* cells, which hydrolyzes phosphodiester bonds in the phospholipid backbone, which leads to the conversion of TAG to DAG (Santi et al., 2010). In previously published studies (Litwin et al., 2021) in the presence of pyrethroids added at a very high concentration of 100 mg L^{-1}, the DAG content exceeded the TAG content in 48-h cultures. The content of TAG in cells also plays an important role in protecting cells from oxidative stress. As reported by Fan et al. (2017), the accumulation of TAGs prevents the formation of ROS, which is consistent with the results of the presented research. It is worth noting that acylglycerols are also essential for fungal virulence, increasing insect epidermal penetration (Gao et al., 2016).

During these studies, individual TAGs and DAG species were also identified and it was shown that the accumulated pyrethroids caused significant changes in the content of individual TAGs and DAGs. The collected data set is in line with our previous findings, which suggest that the observed effects of pyrethroids on TAG and DAG species depend on the substance used (Litwin et al., 2021).

5. Conclusions

In summary, this study reports for the first time that entomopathogenic fungi *Beauveria bassiana* accumulates pyrethroids very efficiently and only approximately 1% and 0.6% remain in the culture medium after 2 and 7 days of incubation, respectively. The effect of accumulated substances on fungal cells varies depending on the type of pyrethroid and in general affects the activity of both extra- and intracellular enzymes in many different ways. Additionally, the effect of accumulated pyrethroids on enzymes involved in basic metabolic pathways and the infectious process was observed. Furthermore, it was shown that the accumulated insecticides caused elevated levels of superoxide anion content. The results of these studies help to explain how accumulated pyrethroids in very large quantities affect fungal metabolism. Our findings may also have practical implications, as both pesticides and entomopathogenic fungi are present in the environment: entomopathogenic fungi occur naturally in soil and are additionally introduced in IPM programs, while pyrethroids remain in the environment after application. Thus, the results of this study seem to be also important from an environmental perspective, since the ability of entomopathogenic fungi to accumulate such high concentrations of insecticides can not only impair their function, but also cause the deposition of these compounds in fungal cells in the environment, contributing to its pollution.

CRediT authorship contribution statement

Anna Litwin: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing. **Julia Mironenka:** Methodology, Validation, Investigation. **Przemysław Bernat:** Methodology, Validation, Investigation. **Adrian Soboń:** Methodology, Validation. **Sylwia Różalska:** Project administration, Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sylwia Rozalska reports financial support was provided by National Science Centre Poland.

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114418.

References

Abbas, M.A., Iqbal, M., Tauqeer, H.M., et al., 2022. Microcontaminants in wastewater. : Environ. Micro 315–329.

Agrawal, T., Kotasthane, A.S., 2012. Chitinolytic assay of indigenous Trichoderma isolates collected from different geographical locations of Chhattisgarh in Central India. Springerplus 1, 1–10.

Barman, A., Gohain, D., Bora, U., Tamuli, R., 2018. Phospholipases play multiple cellular roles including growth, stress tolerance, sexual development, and virulence in fungi. Microbiol Res 209, 55–69. https://doi.org/10.1016/j.micres.2017.12.012.

Behie, S.W., Bidochka, M.J., 2014. Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: An additional branch of the soil nitrogen cycle. Appl. Environ. Microbiol 80, 1553–1560. https://doi.org/10.1128/AEM.03338-13.

Bernat, P., Nykiel-Szymańska, J., Stolarek, P., et al., 2018. 2,4- dichlorophenoxyacetic acid-induced oxidative stress: Metabolome and membrane modifications in Umbelopsis isabellina, a herbicide degrader. PLoS One 13.

Bhatt, P., Huang, Y., Zhan, H., Chen, S., 2019. Insight into microbial applications for the biodegradation of pyrethroid insecticides. Front Microbiol 10, 1–19. https://doi.org/10.3389/fmicb.2019.01778.

Bilen, S., Turan, V., 2022. Enzymatic analyses in soils. : Pract. Handb. Agric. Microbiol. 377–385.

Boysen, J.M., Saeed, N., Wolf, T., et al., 2021. The peroxiredoxin asp f3 acts as redox sensor in Aspergillus fumigatus. Genes (Basel) 12. https://doi.org/10.3390/genes12050668.

Breitenbach, M., Weber, M., Rinnerthaler, M., et al., 2015. Oxidative stress in fungi: Its function in signal transduction, interaction with plant hosts, and lignocellulose degradation. Biomolecules 5, 318–342. https://doi.org/10.3390/biom5020318.

Cycoń, M., Piotrowska-Seget, Z., 2016. Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review. Front. Microbiol. 7, 1–26. https://doi.org/10.3389/fmicb.2016.01463.

Dhar, P., Kaur, G., 2010. Cuticle-degrading proteases produced by metarhizium anisopliae and their induction in different media. Indian J. Microbiol 50, 449–455. https://doi.org/10.1007/s12088-011-0098-1.

Djordjevic, J.T., 2010. Role of phospholipases in fungal fitness, pathogenicity, and drug development - lessons from Cryptococcus neoformans. Front Microbiol 1, 1–13. https://doi.org/10.3389/fmicb.2010.00125.

Fan, J., Yu, L., Xu, C., 2017. A central role for triacylglycerol in membrane lipid breakdown, fatty acid β-oxidation, and plant survival under extended darkness. Plant Physiol. 174, 1517–1530. https://doi.org/10.1104/pp.17.00653.

Forlani, L., Juárez, M.P., Lavarías, S., Pedrini, N., 2014. Toxicological and biochemical response of the entomopathogenic fungus Beauveria bassiana after exposure to deltamethrin. Pest Manag Sci. 70, 751–756. https://doi.org/10.1002/ps.3583.

Gangola, S., Sharma, A., Bhatt, P., et al., 2018. Presence of esterase and laccase in Bacillus subtilis facilitates biodegradation and detoxification of cypermethrin. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-31082-5.
Gao, B.J., Mou, Y.N., Tong, S.M., et al., 2020. Subtilisin-like Pr1 proteases marking the evolution of pathogenicity in a wide-spectrum insect-pathogenic fungus. Virulence 11, 365–380. https://doi.org/10.1080/21505594.2020.1749487.
Gao, Q., Lu, Y., Yao, H., et al., 2016. Phospholipid homeostasis maintains cell polarity, development and virulence in Metarhizium robertsii. Environ. Microbiol 18, 3976–3990. https://doi.org/10.1111/1462-2920.13408.
Góralczyk-Binkowska, A., Jasinska, A., Dlugonski, A., et al., 2020. Laccase activity of the ascomycete fungus Nectriella pironii and innovative strategies for its production on leaf litter of an urban park. PLoS One 15, 1–18. https://doi.org/10.1371/journal.pone.0231453.
Hausladen, A., Gow, A.J., Stamler, J.S., 1998. Nitrosative stress: Metabolic pathway involving the flavohemoglobin. Proc. Natl. Acad. Sci. USA 95, 14100–14105. https://doi.org/10.1073/pnas.95.24.14100.
Huarte-Bonnet, C., Juárez, M.P., Pedrini, N., 2015. Oxidative stress in entomopathogenic fungi grown on insect-like hydrocarbons. Curr. Genet 61, 289–297. https://doi.org/10.1007/s00294-014-0452-z.
Khalil, M., Iqbal, M., Turan, V., et al., 2022. Household chemicals and their impact. : Environ. Micro 201–232.
Litwin, A., Nowak, M., Różalska, S., 2020. Entomopathogenic fungi: unconventional applications. Rev. Environ. Sci. Biotechnol. 19, 23–42. https://doi.org/10.1007/s11157-020-09525-1.
Litwin, A., Bernat, P., Nowak, M., et al., 2021. Lipidomic response of the entomopathogenic fungus Beauveria bassiana to pyrethroids. Sci. Rep. 11, 1–11. https://doi.org/10.1038/s41598-021-00702-y.
Liu, J.J., Sturrock, R., Ekramoddoullah, A.K.M., 2010. The superfamily of thaumatin-like proteins: Its origin, evolution, and expression towards biological function. Plant Cell Rep. 29, 419–436. https://doi.org/10.1007/s00299-010-0826-8.
Lu, Z., Deng, J., Wang, H., et al., 2021. Multifunctional role of a fungal pathogen-secreted laccase 2 in evasion of insect immune defense. Environ. Microbiol 23, 1256–1274. https://doi.org/10.1111/1462-2920.15378.
Łaniewska-Dunaj, M., Jelski, W., Szmitkowski, M., 2013. Dehydrogenaza alkoholowa - Znaczenie fizjologiczne i diagnostyczne. Post. Hig. Med. Dosw 67, 901–907. https://doi.org/10.5604/17322693.1064330.
Łopusiewicz, Ł., Mazurkiewicz-Zapałowicz, K., Tkaczuk, C., Bartkowiak, A., 2020. The influence of cobalt ions on growth and enzymatic activity of entomopathogenic fungi used in biological plant protection. J. Plant Prot. Res 60, 58–67. https://doi.org/10.24425/jppr.2020.132207.
Martínková, L., Veselá, A.B., Rinágelová, A., Chmátal, M., 2015. Cyanide hydratases and cyanide dihydratases: emerging tools in the biodegradation and biodetection of cyanide. Appl. Microbiol Biotechnol. 99, 8875–8882. https://doi.org/10.1007/s00253-015-6899-0.
Mayerhofer, J., Enkerli, J., Zelger, R., Strasser, H., 2015. Biological control of the European cockchafer: persistence of Beauveria brongniartii after long-term applications in the Euroregion Tyrol. BioControl 60, 617–629. https://doi.org/10.1007/s10526-015-9671-6.
Mironenka, J., Różalska, S., Soboń, A., Bernat, P., 2021. Trichoderma harzianum metabolites disturb Fusarium culmorum metabolism: metabolomic and proteomic studies. Microbiol Res 249.
Mondal, S., Baksi, S., Koris, A., Vatai, G., 2016. Journey of enzymes in entomopathogenic fungi. Pac. Sci. Rev. A Nat. Sci. Eng. 18, 85–99. https://doi.org/10.1016/j.psra.2016.10.001.
Morel, M., Ngadin, A.A., Droux, M., et al., 2009. The fungal glutathione S-transferase system. Evidence of new classes in the wood-degrading basidiomycete Phanerochaete chrysosporium. Cell Mol. Life Sci. 66, 3711–3725. https://doi.org/10.1007/s00018-009-0104-5.
Nowak, M., Soboń, A., Litwin, A., Różalska, S., 2019. 4-n-nonylphenol degradation by the genus Metarhizium with cytochrome P450 involvement. Chemosphere 220, 324–334. https://doi.org/10.1016/j.chemosphere.2018.12.114.
Nowak, M., Bernat, P., Mrozińska, J., Rózalska, S., 2020. Acetamiprid Affects Destruxins Production but Its Accumulation in Metarhizium sp. Spores Increases Infection Ability of Fungi. Toxins (Basel) 12. https://doi.org/10.3390/toxins12090587.
Nykiel-Szymańska, J., Różalska, S., Bernat, P., Słaba, M., 2019. Assessment of oxidative stress and phospholipids alterations in chloroacetanilides-degrading Trichoderma spp. Ecotoxicol. Environ. Saf. 1861, 316–326.
Oide, S., Tanaka, Y., Watanabe, A., Inui, M., 2019. Carbohydrate-binding property of a cell wall integrity and stress response component (WSC) domain of an alcohol oxidase from the rice blast pathogen Pyricularia oryzae. Enzym. Micro Technol. 125, 13–20.
Oliveira, D.G.P., Cardoso, R.R., Mamprim, A.P., Angeli, L.F., 2016. Laboratory and field evaluation of a cypermethrin-based insecticide for the control of Alphitobius diaperinus panzer (Coleoptera: Tenebrionidae) and its in-vitro effects on beauveria bassiana bals. vuill. (hypocreales: Cordycipitaceae). Rev. Bras. Cienc. Avic. 18, 371–380. https://doi.org/10.1590/1806-9061-2015-0115.
Orlando Beys Silva, W., Mitidieri, S., Schrank, A., Vainstein, M.H., 2005. Production and extraction of an extracellular lipase from the entomopathogenic fungus Metarhizium anisopliae. Process Biochem 40, 321–326. https://doi.org/10.1016/j.procbio.2004.01.005.
Perinotto, W.M.S., Golo, P.S., Coutinho Rodrigues, C.J.B., et al., 2014. Enzymatic activities and effects of mycovirus infection on the virulence of Metarhizium anisopliae in Rhipicephalus microplus. Vet. Parasitol. 203, 189–196. https://doi.org/10.1016/j.vetpar.2014.02.011.
Rasool, B., Mahmood-ur-Rahman, Zubair, M., et al., 2022. Synergetic efficacy of amending Pb-polluted soil with P-loaded jujube (ziziphus mauritiana) twigs biochar and foliar chitosan application for reducing Pb distribution in moringa leaf extract and improving its anti-cancer potential. Water Air Soil Pollut. 233. https://doi.org/10.1007/s11270-022-05807-2.
Rocha, M.C., de Godoy, K.F., Bannitz-Fernandes, R., et al., 2018. Analyses of the three 1-Cys Peroxiredoxins from Aspergillus fumigatus reveal that cytosolic Prx1 is central to H2O2 metabolism and virulence. Sci. Rep. 8, 1–18. https://doi.org/10.1038/s41598-018-30108-2.
Santi, L., Beys da Silva, W.O., Berger, M., et al., 2010. Conidial surface proteins of Metarhizium anisopliae: source of activities related with toxic effects, host penetration and pathogenesis. Toxicon 55, 874–880. https://doi.org/10.1016/j.toxicon.2009.12.012.
Soboń, A., Szewczyk, R., Długoński, J., Różalska, S., 2019. A proteomic study of Cunninghamella echinulata recovery during exposure to tributyltin. Environ. Sci. Pollut. Res 26, 32545–32558. https://doi.org/10.1007/s11356-019-06416-z.
Stolarek, P., Różalska, S., Bernat, P., 2019. Lipidomic adaptations of the Metarhizium robertsii strain in response to the presence of butyltin compounds. Biochim Biophys. Acta - Biomembr. 1861, 316–326. https://doi.org/10.1016/j.bbamem.2018.06.007.
Sun, M., Ren, Q., Liu, Z., et al., 2011. Beauveria bassiana: Synergistic effect with acaricides against the tick Hyalomma anatolicum anatolicum (Acari: Ixodidae). Exp. Parasitol. 128, 192–195.
Szewczyk, R., Soboń, A., Słaba, M., Długoński, J., 2015. Mechanism study of alachlor biodegradation by Paecilomyces marquandii with proteomic and metabolomic methods. J. Hazard Mater. 291, 52–64.
Tang, W., Wang, D., Wang, J., et al., 2018. Pyrethroid pesticide residues in the global environment: An overview. Chemosphere 191, 990–1007. https://doi.org/10.1016/j.chemosphere.2017.10.115.
Tauqeer, H.M., Basharat, Z., Adnan Ramzani, P.M., et al., 2022. Aspergillus niger-mediated release of phosphates from fish bone char reduces Pb phytoavailability in Pb-acid batteries polluted soil, and accumulation in fenugreek. Environ. Pollut. 313. https://doi.org/10.1016/j.envpol.2022.120064.
Turan, V., 2021. Arbuscular mycorrhizal fungi and pistachio husk biochar combination reduces Ni distribution in mungbean plant and improves plant antioxidants and soil enzymes. Physiol. Plant 173, 418–429. https://doi.org/10.1111/ppl.13490.
Turan, V., 2022. Calcite in combination with olive pulp biochar reduces Ni mobility in soil and its distribution in chili plant. Int J. Phytoremediat. 24, 166–176. https://doi.org/10.1080/15226514.2021.1929826.
Wang, J., Chen, J., Hu, Y., et al., 2020. Roles of six Hsp70 genes in virulence, cell wall integrity, antioxidant activity and multiple stress tolerance of Beauveria bassiana. Fungal Genet. Biol. 144.
Wang, P., Heitman, J., 2005. The cyclophilins. Genome Biol. 6. https://doi.org/10.1186/gb-2005-6-7-226.
Xu, L., Ferry, N., Wang, Z., et al., 2013. A proteomic approach to study the mechanism of tolerance to Bt toxins in Ostrinia furnacalis larvae selected for resistance to Cry1Ab. Transgenic Res. 22, 1155–1166. https://doi.org/10.1007/s11248-013-9718-3.
Zhao, Y.C., Su, L., Li, K., Zhao, B.X., 2020. Discovery of novel PC-PLC activity inhibitors. Chem. Biol. Drug Des. 95, 380–387.

Artykuł naukowy P3 – *Supplementary*

Accumulation of pyrethroids induces changes in metabolism of the entomopathogenic fungus *Beauveria bassiana*—proteomic and lipidomic background

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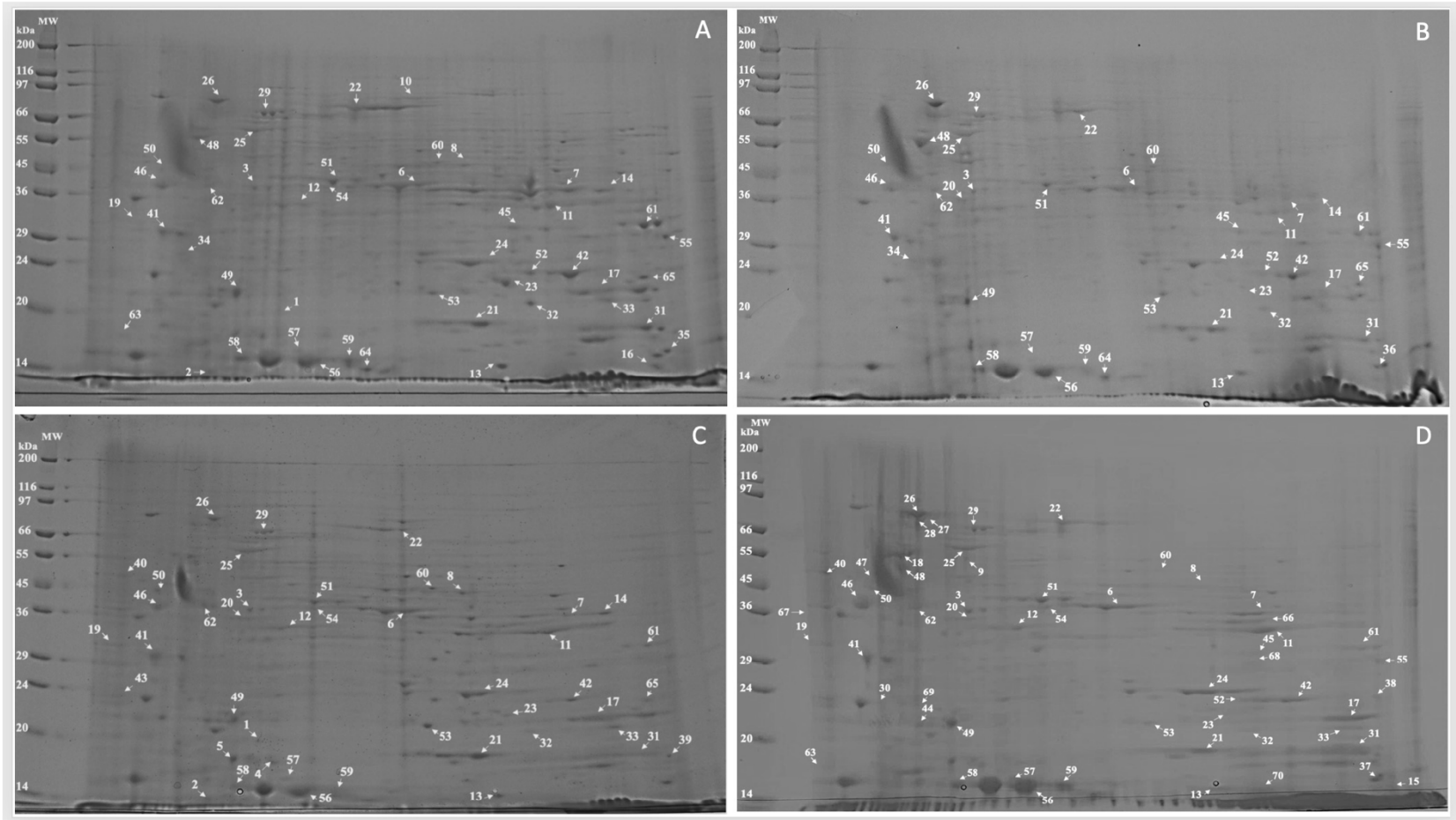


Supplementary Fig. S1. 2-D SDS–PAGE protein profile of the 48-h *B. bassiana* biotic control (A), cultures with accumulated λ-cyhalothrin (B), α-cypermethrin (C) and deltamethrin (D).

Supplementary Table S1. Set of intracellular proteins (determined by MALDI TOF/TOF after 2-D SDS–PAGE electrophoresis) in 48-h *B. bassiana* cultures with accumulated λ-cyhalothrin, α-cypermethrin, and deltamethrin.

Spot no	Accession no[a]	Theoretical mass [Da]	Practical mass [kDa]	Score[b]	Protein name	Species homology	Spot intensity[c]			
							Biotic control	Cyh[d]	Cyp[e]	Delt[f]
Nitrogen compound metabolic process										
1	EJP65399.1	16499	19	110	Cyanate hydratase	*Beauveria bassiana*	10	-	24	-
Cytoskeleton proteins										
2	KAF1736820.1	9182	13	128	Profilin-2	*Beauveria bassiana*	33	-	23	-
3	EJP62356.1	35262	36	679	Bin/amphiphysin/Rvs domain for vesicular trafficking	*Beauveria bassiana* ARSEF 2860	17	14	17	34
4	KGQ08562.1	16668	17	153	Cofilin	*Beauveria bassiana* D1-5	-	-	19	-
5	KGQ08562.1	16668	17	84	Cofilin	*Beauveria bassiana* D1-5	-	-	28	-
Glycolysis										
6	PMB72521.1	39286	39	610	Fructose-bisphosphate aldolase	*Beauveria bassiana*	20	13	24	36
7	AAT80324.1	36069	36	237	Glyceraldehyde-3-phosphate dehydrogenase	*Beauveria bassiana*	23	12	32	30
8	EJP63682.1	44282	45	93	Phosphoglycerate kinase	*Beauveria bassiana* ARSEF 2860	17	-	20	14
9	KGQ02570.1	47244	53	160	Enolase	*Beauveria bassiana* D1-5	-	-	-	14
The citric acid cycle										
10	KAF1737033	85422	84	84	Aconitate hydratase	*Beauveria bassiana* D1-5	14	-	-	-
11	EJP64824.1	34318	34	764	Malate dehydrogenase	*Beauveria bassiana* ARSEF 2860	21	12	18	43
The pentose phosphate cycle										

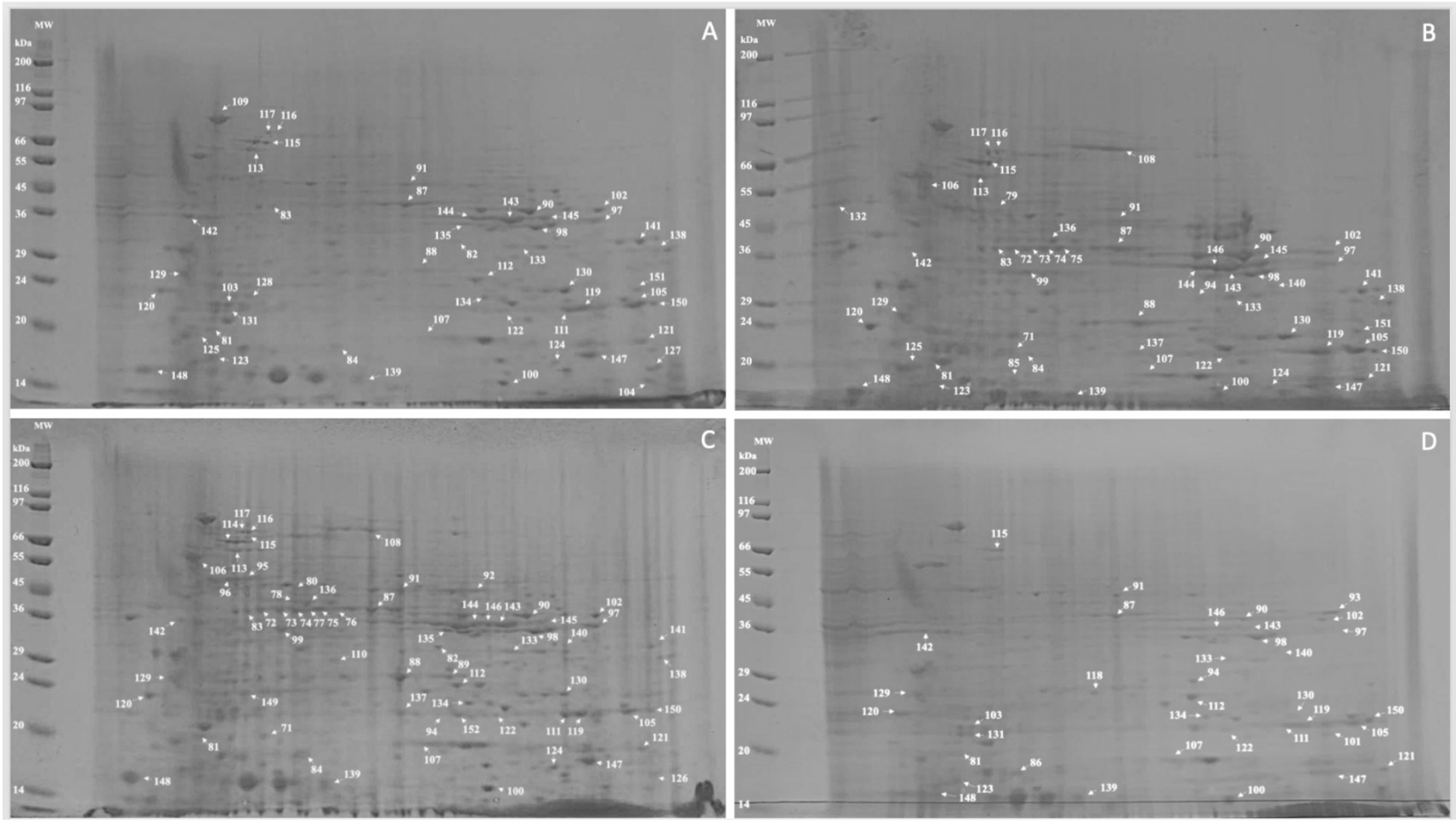
12	KAF1735528.1	35186	34	339	Transaldolase	*Beauveria bassiana*	11	-	13	27
					Electron transport chain					
13	EJP62193.1	13672	14	84	NADH-ubiquinone reductase complex 1 MLRQ subunit; T	*Beauveria bassiana* ARSEF 2860	36	16	27	46
14	EJP70615.1	37613	36	107	Oxidoreductase NAD-binding domain-containing protein [Beauveria bassiana ARSEF 2860]	*Beauveria bassiana* ARSEF 2860	21	12	21	-
15	EJP65387.1.	11454	15	70	Cytochrome C	*Beauveria bassiana* ARSEF 2860	-	-	-	25
16	KAF1730116.1	14263	14	239	Cytochrome b-c1 complex subunit 7	*Beauveria bassiana*	25	-	-	-
17	EJP61409.1	19305	21	182	ATP synthase subunit D	*Beauveria bassiana* ARSEF 2860	16	15	19	38
18	EJP62887.1	55284	55	743	ATP synthase beta chain	*Beauveria bassiana* ARSEF 2860	-	-	-	27
					Other proteins involved in energy production					
19	PQK14156.1	33971	31	79	hypothetical protein BB8028_0004g10860 - ADP/ATP transporter on adenylate translocase; Provisional	*Beauveria bassiana*	9	-	21	33
20	EJP66785.1	37494	36	99	Adenosine kinase	*Beauveria bassiana* ARSEF 2860	-	12	11	14
					Stress response					
21	PMB68371.1	15747	17	287	Superoxide disumtase (Cu-Zn)	*Beauveria bassiana*	38	21	37	39
22	KGQ07030.1	73792	75	177	Catalase	*Beauveria bassiana*	23	22	18	44
23	PMB67240.1	24402	22	93	Glutathione S-transferase	*Beauveria bassiana*	31	11	12	13

24	EJP70971.1	24745	25	317	Mitochondrial peroxiredoxin PRX1	*Beauveria bassiana* ARSEF 2860	17	12	20	47
25	KAF1733028.1	61567	60	784	Heat shock protein 60	*Beauveria bassiana*	20	17	23	31
26	ACC63375.1	71251	79	570	Heat shock protein 70	*Beauveria bassiana*	29	38	24	55
27	ACC63375.1	71251	74	345	Heat shock protein 70	*Beauveria bassiana*	-	-	-	26
28	ACC63375.1	71251	73	330	Heat shock protein 70	*Beauveria bassiana*	-	-	-	26
29	EJP69543.1	73738	72	896	Hsp70-like protein	*Beauveria bassiana* ARSEF 2860	24	15	20	40
30	EJP67847.1	21245	23	63	Hsp90 associated co-chaperone	*Beauveria bassiana* ARSEF 2860	-	-	-	11
Chaoperones and other proteins involved in protein metabolism										
31	OAA38210.1	15774	17	164	Cyclophilin A	*Beauveria brongniartii* RCEF 3172	38	18	16	36
32	OAA45266.1	22684	20	420	Cyclophilin B	*Beauveria brongniartii* RCEF 3172	24	10	9	11
33	AEE02047.1	19628	20	373	Cyclophilin cytosolic form	*Beauveria bassiana*	23	-	16	20
34	KAF1738066.1	28003	26	97	31-O-demethyl-FK506 methyltransferase FkbM	*Beauveria bassiana*	12	15	-	-
35	EJP61827.1	14198	15	351	Ribosomal L22e family protein	*Beauveria bassiana* ARSEF 2860	24	-	-	-
36	EJP61827.1	14198	15	174	Ribosomal L22e family protein	*Beauveria bassiana* ARSEF 2860	-	19	-	-
37	KAF1729983.1	14198	15	110	60S ribosomal protein L22	*Beauveria bassiana*	-	-	-	51
38	EJP62493.1	23112	23	71	Ribosomal protein S7	*Beauveria bassiana* ARSEF 2860	-	-	-	15
39	EJP66868.1	16645	17	99	40S ribosomal protein S19	*Beauveria bassiana* ARSEF 2860	-	-	17	-
40	AAU95328.1	46548	49	127	Translation elongation factor 1 alpha, partial	*Beauveria bassiana*	-	-	16	43
41	EJP69604.1	24900	29	791	Elongation factor 1-beta	*Beauveria bassiana* ARSEF 2860	24	21	21	46

42	PQK15291.1	22856	23	460	hypothetical protein BB8028_0005g08050 [Beauveria bassiana] - eukaryotic initiation factor 5a; Provisional	*Beauveria bassiana*	41	30	22	41
43	PQK15291.1	22856	23	107	Eukaryotic initiation factor 5a	*Beauveria bassiana*	-	-	17	-
44	PMB71240.1	21877	21	132	Eukaryotic translation initiation factor 5A	*Beauveria bassiana*	-	-	-	18
45	PMB67890.1	28602	30	123	Subtilisin-like protease 1	*Beauveria bassiana*	17	14	-	14
46	OAA37714.1	42364	39	465	Vacuolar protease A precursor	*Beauveria brongniartii* RCEF 3172	21	17	22	40
47	EJP66395.1	42349	47	90	Vacuolar protease A precursor	*Beauveria brongniartii* RCEF 3172	-	-	-	20
48	EJP63011.1	55547	56	333	Protein disulfide isomerase	*Beauveria bassiana* ARSEF 2860	19	27	-	18
49	OAA41166.1	19264	21	156	Beta-flanking protein	*Beauveria brongniartii* RCEF 3172	31	22	19	54
50	EJP63931.1	37691	42	90	RNA recognition domain-containing protein	*Beauveria bassiana* ARSEF 2860	12	15	17	24
					Amino acids metabolism					
51	KGQ12327.1	43215	40	806	S-adenosylmethionine synthase	*Beauveria bassiana* D1-5	26	16	27	54
					Riboflavin metabolism					
52	EJP68394.1	22193	23	88	NADPH-dependent FMN reductase	*Beauveria bassiana* ARSEF 2860	19	10	-	20
53	PMB70661.1	21403	21	281	Minor allergen Alt a 7	*Beauveria bassiana*	18	17	29	19
					Other					
54	EJP64883.1	37620	38	131	Esterase-like protein	*Beauveria bassiana* ARSEF 2860	12	-	18	28
55	EJP64384.1	25732	29	209	Vacuolar ATP synthase subunit E	*Beauveria bassiana* ARSEF 2860	23	18	-	26

56	EJP64445.1	13823	14	599	CipC protein	*Beauveria bassiana* ARSEF 2860	51	39	47	77
57	EJP64445.1	13823	15	370	CipC protein	*Beauveria bassiana* ARSEF 2860	15	10	16	35
58	EJP64445.1	13823	14	150	CipC protein	*Beauveria bassiana* ARSEF 2860	29	24	16	37
59	EJP64445.1	13823	14	400	CipC protein	*Beauveria bassiana* ARSEF 2860	32	14	17	37
60	EJP61302.1	52860	47	289	Woronin body major protein	*Beauveria bassiana* ARSEF 2860	16	13	25	9
61	OAA37935.1	29958	31	421	Outer mitochondrial membrane protein porin	*Beauveria brongniartii* RCEF 3172	39	14	15	17
62	EJP62392.1	39234	39	200	Tetratricopeptide-like protein	*Beauveria bassiana* ARSEF 2860	15	10	17	14
63	EJP68986.1	16931	17	161	Nucleoside diphosphate kinase	*Beauveria bassiana* ARSEF 2860	11	-	-	22
64	EJP67324.1	14925	14	100	Small secreted protein	*Beauveria bassiana* ARSEF 2860	31	22	-	-
65	EJP69326.1	22084	22	227	Tim17/Tim22/Tim23 family protein	*Beauveria bassiana* ARSEF 2860	24	17	13	-
66	EJP64991.1	37006	35	150	Alcohol dehydrogenase	*Beauveria bassiana* ARSEF 2860	-	-	-	18
67	PQK12931.1	37006	36	72	hypothetical protein BB8028_0003g15460 - D-arabinose 1-dehydrogenase, Zn-dependent alcohol dehydrogenase family	*Beauveria bassiana*	-	-	-	31
68	KAF1733968.1	24324	28	81	NADP-dependent 3-hydroxy acid dehydrogenase	*Beauveria bassiana*	-	-	-	15
69	KAF1729484.1	19086	23	83	hypothetical protein CRV24_010557 - unknown function	*Beauveria bassiana*	-	-	-	15
70	PMB65047.1	14944	14	102	hypothetical protein BM221_009235 - unknown function	*Beauveria bassiana*	-	-	-	42

[a]Accession no—the protein sequence record ID given by Mascot; [b]Score—cumulative scores for individual peptides for all peptides matching a given protein; [c]Spot intensity—the sum of the pixel intensities within the spot area; [d]Cyh—λ-cyhalothrin; [e]Cyp—α-cypermethrin; [f]Delt—deltamethrin.



Supplementary Fig. S2. 2-D SDS–PAGE protein profile of the 168-h *B. bassiana* biotic control (A), cultures with accumulated λ-cyhalothrin (B), α-cypermethrin (C) and deltamethrin (D).

Supplementary Table S2. Set of intracellular proteins (determined by MALDI TOF/TOF after 2-D SDS–PAGE electrophoresis) in 168-h *B. bassiana* cultures with accumulated λ-cyhalothrin, α-cypermethrin, and deltamethrin.

Spot no	Accession no[a]	Theoretical mass [Da]	Practical mass [kDa]	Score[b]	Protein name	Species homology	Spot intensity[c]			
							Biotic control	Cyh[d]	Cyp[e]	Delt[f]
Nitrogen compound metabolic process										
71	KAF1732782.1	17600	20	483	Cyanate hydratase	*Beauveria bassiana*	12	-	22	-
72	EJP63242.1	42067	39	224	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	24	-	34	-
73	EJP63242.1	42067	39	329	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	29	-	46	-
74	EJP63242.1	42067	39	100	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	31	-	45	-
75	EJP63242.1	42067	39	165	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	27	-	38	-
76	EJP63242.1	42067	39	100	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	-	-	23	-
77	EJP63242.1	42067	38	125	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	-	-	36	-
78	EJP63242.1	42067	41	55	Cyanide hydratase	*Beauveria bassiana* ARSEF 2860	-	-	23	-
79	PMB65117.1	50675	50	129	Flavohemoprotein	*Beauveria bassiana*	15	-	-	-
80	PMB65117.1	50675	46	166	Flavohemoprotein	*Beauveria bassiana*	-	-	22	-
Cytoskeleton proteins										
81	EJP63879.1	18854	19	87	Actin lateral binding protein	*Beauveria bassiana* ARSEF 2860	11	22	20	7
82	EJP65288.1	29659	31	383	F-actin-capping protein subunit alpha	*Beauveria bassiana* ARSEF 2860	-	7	28	-
83	EJP62356.1	35262	38	104	Bin/amphiphysin/Rvs domain for vesicular trafficking	*Beauveria bassiana* ARSEF 2860	22	14	27	-

84	KAF1733027.1	16660	17	314	Cofilin	*Beauveria bassiana* D1-5	8	14	26	-
85	KGQ08562.1	16668	17	69	Cofilin	*Beauveria bassiana* D1-5	22	-	-	-
86	KAF1733027.1	16660	17	364	Cofilin	*Beauveria bassiana*	-	-	-	15
					Glycolysis					
87	EJP62840.1	39229	38	432	Fructose-bisphosphate aldolase	*Beauveria bassiana* ARSEF 2860	33	44	58	36
88	EJP65228.1	26969	26	365	Triosephosphate isomerase	*Beauveria bassiana* ARSEF 2860	38	12	79	-
89	EJP65228.1	26969	25	94	Triosephosphate isomerase	*Beauveria bassiana* ARSEF 2860	-	-	28	-
90	AAT80324.1	36069	36	664	Glyceraldehyde-3-phosphate dehydrogenase	*Beauveria bassiana*	71	57	50	16
91	EJP63682.1	44282	45	106	Phosphoglycerate kinase	*Beauveria bassiana* ARSEF 2860	10	19	47	24
92	KAF1734661.1	44298	46	493	Phosphoglycerate kinase	*Beauveria bassiana*	-	-	23	-
93	EJP63682.1	44282	42	94	Phosphoglycerate kinase	*Beauveria bassiana* ARSEF 2860	-	-	-	23
94	KAF1730274.1	34576	30	337	Enolase	*Beauveria bassiana*	9	-	4	17
95	KGQ02570.1	47244	49	313	Enolase	*Beauveria bassiana* D1-5	-	-	26	-
96	KGQ02570.1	47244	49	163	Enolase	*Beauveria bassiana* D1-5	-	-	24	-
					The citric acid cycle					
97	KGQ07683.1	35420	36	413	Putative succinyl-CoA ligase [ADP-forming] subunit alpha	*Beauveria bassiana* D1-5	22	27	38	6
98	KAF1732961.1	34036	34	551	Malate dehydrogenase	*Beauveria bassiana*	49	56	66	34
					The pentose phosphate cycle					
99	OAA40205.1	35580	34	259	Transaldolase	*Beauveria bassiana*	26	-	35	-

					Electron transport chain (ETC)					
100	EJP62193.1	13672	14	307	hypothetical protein BBA_08867 - NADH-ubiquinone reductase complex 1 MLRQ subunit;	*Beauveria bassiana* ARSEF 2860	46	58	71	71
101	EJP67717.1	22550	21	308	NADH-ubiquinone oxidoreductase 21kDa subunit	*Beauveria bassiana* ARSEF 2860	-	-	-	10
102	KAF1736569.1	37613	39	316	NADH-cytochrome b5 reductase 2	*Beauveria bassiana*	24	41	49	14
103	EJP62228.1	22504	22	115	Cytochrome c oxidase polypeptide IV	*Beauveria bassiana* ARSEF 2860	-	33	-	16
104	KAF1730116.1	14263	14	236	Cytochrome b-c1 complex subunit 7	*Beauveria bassiana*	-	29	-	-
105	EJP61409.1	19305	21	370	ATP synthase subunit D	Beauveria bassiana ARSEF 2860	65	67	52	26
106	EJP62887.1	55284	53	1050	ATP synthase beta chain	*Beauveria bassiana* ARSEF 2860	30	-	43	-
					Stress response					
107	ACT75665.1	15731	18	177	Cu/Zn superoxide dismutase	*Beauveria bassiana*	22	14	28	15
108	AFP20274.1	84151	80	457	Catalase P	*Beauveria bassiana*	40	-	44	-
109	AFP20276.1	87299	92	67	Catalase-peroxidase	*Beauveria bassiana*	-	19	-	-
110	PMB70210.1	30574	29	139	Glutathione S-transferase	*Beauveria bassiana*	-	-	12	-
111	EJP68591.1	19095	21	81	Glutathione peroxidase	*Beauveria bassiana* ARSEF 2860	-	44	59	12
112	OAA45933.1	24658	25	339	Peroxiredoxin-6	*Beauveria brongniartii* RCEF 3172	-	33	40	32
113	KAF1733028.1	61567	63	84	Heat shock protein 60	*Beauveria bassiana*	23	16	24	-
114	OAA44479.1	66544	67	149	Heat shock protein 70	*Beauveria brongniartii* RCEF 3172	-	-	37	-

115	EJP70627.1	66558	64	129	Hsp70-like protein	*Beauveria bassiana* ARSEF 2860	53	36	20	5
116	KAF1737353.1	73086	74	315	Heat shock protein	*Beauveria bassiana*	33	10	32	-
117	KAF1737353.1	73086	74	280	Heat shock protein	*Beauveria bassiana*	37	23	22	-
118	EJP62362.1	21770	26	407	Heat shock protein 30	*Beauveria bassiana* ARSEF 2860	-	-	-	14
119	OAA38998.1	20464	21	71	Carbohydrate-binding WSC domain protein	*Beauveria brongniartii* RCEF 3172	60	46	61	15
Chaperones and other proteins involved in protein metabolism										
120	KGQ04193.1	21231	23	432	Protein wos2	*Beauveria bassiana* D1-5	60	32	41	18
121	AAN39296.1	17520	18	94	Cyclophilin A	*Beauveria bassiana*	48	28	22	10
122	KAF1733512.1	21434	21	65	Peptidyl-prolyl cis-trans isomerase ssp-1	*Beauveria bassiana*	26	18	16	10
123	AFS18601.1	14769	16	69	FK506-binding protein 2	*Beauveria bassiana*	23	21	-	12
124	KAF1731705.1	14363	16	138	37S ribosomal protein YMR-31, mitochondrial	*Beauveria bassiana*	29	18	39	-
125	EJP66933.1	18685	19	106	40S ribosomal protein S15	*Beauveria bassiana* ARSEF 2860	26	16	-	-
126	EJP70230.1	14834	16	77	40S ribosomal protein S22	*Beauveria bassiana* ARSEF 2860	-	-	19	-
127	EJP61827.1	14198	15	82	Ribosomal L22e family protein	*Beauveria bassiana* ARSEF 2860	-	33	-	-
128	OAA42805.1	20869	22	156	Nuclear movement protein nudC	*Beauveria brongniartii* RCEF 3172	-	15	-	-
129	EJP69604.1	24900	25	138	Elongation factor 1-beta	*Beauveria bassiana* ARSEF 2860	22	26	28	17
130	PQK15291.1	22856	23	323	hypothetical protein BB8028_0005g08050 - eukaryotic initiation factor 5a; Provisional	*Beauveria bassiana*	62	59	36	6
131	PMB71240.1	21877	21	152	Eukaryotic translation initiation factor 5A	*Beauveria bassiana*	-	29	-	16

132	AAU95290.1	46527	52	64	Translation elongation factor 1 alpha, partial	*Beauveria bassiana*	29	-	-	-
133	PMB67890.1	28602	30	115	Subtilisin-like protease 1	*Beauveria bassiana*	21	14	25	7
134	EJP70487.1	19122	22	65	RNA recognition domain-containing protein	*Beauveria bassiana* ARSEF 2860	-	17	35	12
135	KGQ07149.1	35598	34	144	Protein vip1	*Beauveria bassiana* D1-5	-	20	45	-
					Amino acids metabolism					
136	KGQ12327.1	43215	41	299	S-adenosylmethionine synthase	*Beauveria bassiana* D1-5	35	-	24	-
					Riboflavin metabolism					
137	KAF1737441.1	21389	21	168	Minor allergen Alt a 7	*Beauveria bassiana*	12	-	28	-
					Other					
138	EJP64384.1	25732	29	163	Vacuolar ATP synthase subunit E	*Beauveria bassiana* ARSEF 2860	11	15	22	-
139	EJP64445.1	13823	14	238	CipC protein	*Beauveria bassiana* ARSEF 2860	62	20	33	45
140	PMB68322.1	38702	31	97	Woronin body major protein	*Beauveria bassiana* ARSEF 2860	11	-	24	5
141	OAA37935.1	29958	30	375	Outer mitochondrial membrane protein porin	*Beauveria brongniartii* RCEF 3172	43	46	21	-
142	EJP64991.1	37006	36	319	Alcohol dehydrogenase I	*Beauveria bassiana* ARSEF 2860	13	36	25	13
143	EJP64991.1	37006	37	311	Alcohol dehydrogenase I	*Beauveria bassiana* ARSEF 2860	51	52	53	13
144	EJP64991.1	37006	35	926	Alcohol dehydrogenase I	*Beauveria bassiana* ARSEF 2860	58	32	57	11
145	EJP64991.1	37006	35	601	Alcohol dehydrogenase I	*Beauveria bassiana* ARSEF 2860	39	25	21	-
146	EJP64991.1	37006	35	776	Alcohol dehydrogenase I	*Beauveria bassiana* ARSEF 2860	55	-	33	11
147	EJP68986.1	16931	17	378	Nucleoside diphosphate kinase	*Beauveria bassiana* ARSEF 2860	52	58	67	19

148	PMB67886.1	14538	15	239	hypothetical protein BM221_006061 - Ricin-type beta-trefoil	*Beauveria bassiana*	56	35	49	23
149	KAF1729630.1	24848	23	74	Rho GDP-dissociation inhibitor	*Beauveria bassiana*	-	-	22	-
150	EJP68212.1	19010	21	211	hypothetical protein BBA_03108 - unknown function	*Beauveria bassiana* ARSEF 2860	36	25	23	26
151	EJP65059.1	21653	23	71	hypothetical protein BBA_05829 - unknown function	*Beauveria bassiana* ARSEF 2860	25	19	-	-
152	PMB66894.1	18994	21	123	hypothetical protein BM221_006551- unknown function	*Beauveria bassiana*	-	-	31	-

[a]Accession no—the protein sequence record ID given by Mascot; [b]Score—cumulative scores for individual peptides for all peptides matching a given protein; [c]Spot intensity—the sum of the pixel intensities within the spot area; [d]Cyh—λ-cyhalothrin; [e]Cyp—α-cypermethrin; [f]Delt—deltamethrin.

Supplementary Table S3. Phospholipase C intracellular [μMol min^{-1}] activity of *B. bassiana* with accumulated pyrethroids.

	Pyrethroids (5 mg L^{-1})			
	Biotic control	λ-cyhalothrin	α-cypermethrin	Deltamethrin
48 h	0.240 ± 0.021	0.181 ± 0.002**	0.194 ± 0.024**	0.201 ± 0.029**
120 h	0.231 ± 0.030	0.278 ± 0.022**	0.243 ± 0.027	0.217 ± 0.010
168 h	0.252 ± 0.008	0.276 ± 0.022	0.220 ± 0.015**	0.228 ± 0.014**

Data are expressed as means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice; n = 6.
Results with a normal distribution were tested using one-way analysis of variance, whereas nonparametric Kruskal–Wallis test was used to analyze the results that were not normally distributed; significance: **—$p < 0.01$, *—$p < 0.05$.

Supplementary Table S4. Percentage content of tri- and diacylglycerols in *B. bassiana* cultures with accumulated λ-cyhalothrin, α-cypermethrin, and deltamethrin.

		Pyrethroids (5 mg L^{-1})			
		Control	λ –cyhalothrin	α –cypermethrin	Deltamethrin
24 h	TAG 48:0	1.74 ± 1.38	0.50 ± 0.07	0.44 ± 0.09*	0.53 ± 0.05
	TAG 50:0	0.67 ± 0.52	0.1 ± 0.03**	0.26 ± 0.04	0.24 ± 0.04
	TAG 50:1	2.11 ± 0.69	1.23 ± 0.12	1.43 ± 0.01	1.16 ± 0.12*
	TAG 52:1	1.37 ± 0.05	1.43 ± 0.28	1.45 ± 0.04	1.48 ± 0.10
	TAG 52:2	3.48 ± 0.31	2.05 ± 1.70	3.74 ± 0.53	4.17 ± 0.10
	TAG 52:3	3.64 ± 0.66	5.05 ± 0.16	5.64 ± 0.28**	4.51 ± 0.84
	TAG 52:4	3.96 ± 2.00	4.35 ± 0.56	6.64 ± 1.22*	4.52 ± 0.40
	TAG 54:0	0.22 ± 0.04	0.08 ± 0.01	0.18 ± 0.12	0.23 ± 0.14
	TAG 54:1	0.32 ± 0.21	0.60 ± 0.09	0.61 ± 0.25	0.61 ± 0.13
	TAG 54:2	1.42 ± 0.78	1.71 ± 1.32	2.03 ± 0.15	2.43 ± 0.08
	TAG 54:3	6.26± 1.35	5.82 ± 0.87	4.50 ± 0.59*	5.89 ± 0.35
	TAG 54:4	7.25 ± 2.98	8.33 ± 0.15	7.35 ± 0.42	8.09 ± 0.44
	TAG 54:6	3.77 ± 2.14	4.54 ± 0.66	11.42 ± 1.87**	4.67± 0.64
	DAG 32:0	23.50 ± 13.27	37.51 ± 0.86	30.90 ± 1.36	35.28 ± 1.47
	DAG 34:0	1.08 ± 0.85	0.57 ± 0.02	0.53 ± 0.13	0.49 ± 0.05
	DAG 34:1	3.97 ± 3.63	7.71 ± 0.66	6.39 ± 0.39	7.80 ± 0.58*
	DAG 34:2	2.02 ± 1.56	1.97 ± 0.15	1.65 ± 0.21	1.93 ± 0.28
	DAG 36	0.96 ± 0.73	1.33 ± 0.13	1.09 ± 0.10	1.34 ± 0.06
	DAG 36:1	12.04 ± 5.36	11.22 ± 0.13	10.38 ± 0.65	10.99 ± 0.87
	DAG 36:2	17.36 ± 15.00	3.24 ± 0.31	2.81 ± 0.20**	2.98 ± 0.18
	DAG 36:3	0.75 ± 0.83	0.31 ± 0.05	0.30 ± 0.04	0.34 ± 0.10
	DAG 36:4	2.12 ± 1.98	0.27 ± 0.06*	0.27 ± 0.02*	0.30 ± 0.03
48 h	TAG 48:0	0.51 ± 0.23	0.34 ± 0.01	0.42 ± 0.20	0.22 ± 0.03**
	TAG 50:0	0.21 ± 0.04	0.19 ± 0.01	0.19 ± 0.04	0.19 ± 0.02
	TAG 50:1	1.24 ± 0.37	1.05 ± 0.05	1.12 ± 0.17	1.11 ± 0.22
	TAG 52:1	0.51 ± 0.15	0.51 ± 0.02	0.51 ± 0.08	0.53 ± 0.07
	TAG 52:2	1.31 ± 0.47	1.21 ± 0.14	1.35 ± 0.26	1.37 ± 0.12
	TAG 52:3	1.29 ± 0.33	1.56 ± 0.15	1.77 ± 0.23	1.84 ± 0.23**
	TAG 52:4	1.28 ± 0.41	1.92 ± 0.13	2.36 ± 0.45	2.25 ± 0.21
	TAG 54:0	0.06 ± 0.01	0.04 ± 0.03	0.07 ± 0.00	0.04 ± 0.02
	TAG 54:1	0.12 ± 0.03	0.05 ± 0.05	0.04 ± 0.02	0.01 ± 0.00**
	TAG 54:2	0.36 ± 0.24	0.60 ± 0.10	0.70 ± 0.17	0.64 ± 0.11

	TAG 54:3	2.60 ± 0.54	2.67 ± 0.11	2.76 ± 0.32	3.06 ± 0.35
	TAG 54:4	3.12 ± 0.81	3.67 ± 0.30	3.90 ± 0.73	3.92 ± 0.28
	TAG 54:6	0.98 ± 0.26	1.16 ± 0.02	1.82 ± 0.15**	1.41 ± 0.13
	DAG 32:0	6.50 ± 0.56	8.21 ± 0.37	6.42 ± 0.95	5.72 ± 0.39
	DAG 34:0	0.24 ± 0.04	0.21 ± 0.03	0.26 ± 0.04	0.20 ± 0.02
	DAG 34:1	0.11 ± 0.08	0.07 ± 0.05	0.78 ± 0.61	0.80 ± 0.08
	DAG 34:2	0.70 ± 0.11	1.05 ± 0.23	0.93 ± 0.37	0.45 ± 0.02
	DAG 36	0.23 ± 0.03	0.21 ± 0.05	0.28 ± 0.05	0.32 ± 0.02
	DAG 36:1	1.59 ± 0.47	1.25 ± 0.13	1.48 ± 0.37	2.07 ± 0.50
	DAG 36:2	2.18 ± 0.32	2.71 ± 0.68	2.33 ± 0.94	1.62 ± 0.21
	DAG 36:3	0.15 ± 0.08	0.23 ± 0.04	0.21 ± 0.05	0.11 ± 0.06
	DAG 36:4	0.49 ± 0.15	0.35 ± 0.03	0.36 ± 0.08	0.26 ± 0.01**
120 h	TAG 48:0	0.28 ± 0.04	0.27 ± 0.01	0.27 ± 0.03	0.27 ± 0.03
	TAG 50:0	0.53 ± 0.06	0.66 ± 0.12	0.69 ± 0.05**	0.62 ± 0.02
	TAG 50:1	2.87 ± 0.11	3.42 ± 0.49*	3.33 ± 0.22	3.37 ± 0.14
	TAG 52:1	1.74 ± 0.08	2.36 ± 0.43**	2.43 ± 0.07**	2.32 ± 0.07**
	TAG 52:2	3.21 ± 0.12	3.89 ± 0.17	4.92 ± 0.30**	3.85 ± 0.34
	TAG 52:3	3.49 ± 0.24	4.75 ± 0.39	4.98 ± 0.72*	4.36 ± 0.86
	TAG 52:4	4.92 ± 0.22	5.64 ± 0.46	8.32 ± 0.22**	5.58 ± 0.27
	TAG 54:0	0.10 ± 0.00	0.15 ± 0.06	0.13 ± 0.01	0.13 ± 0.00
	TAG 54:1	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
	TAG 54:2	2.06 ± 0.19	2.57 ± 0.44	2.58 ± 0.13**	2.40 ± 0.17
	TAG 54:3	4.96 ± 0.50	6.54 ± 0.68	7.60 ± 0.36**	6.64 ± 0.50
	TAG 54:4	6.57 ± 0.14	8.13 ± 0.27	9.52 ± 0.38**	7.81 ± 0.24
	TAG 54:6	3.86 ± 0.36	4.06 ± 0.78	6.60 ± 0.52**	4.90 ± 0.38
	DAG 32:0	16.39 ± 3.25	17.06 ± 1.08	17.39 ± 0.96	18.52 ± 0.38
	DAG 34:0	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.00	0.09 ± 0.02
	DAG 34:1	0.84 ± 0.04	0.45 ± 0.16**	0.80 ± 0.21	0.67 ± 0.23
	DAG 34:2	0.30 ± 0.08	0.25 ± 0.06	0.25 ± 0.03	0.18 ± 0.02*
	DAG 36	0.11 ± 0.05	0.12 ± 0.13	0.12 ± 0.12	0.10 ± 0.15
	DAG 36:1	6.79 ± 1.11	6.73 ± 0.78	6.76 ± 0.78	7.09 ± 0.57
	DAG 36:2	1.51 ± 0.30	1.45 ± 0.05	1.41 ± 0.05	1.19 ± 0.09*
	DAG 36:3	0.10 ± 0.04	0.11 ± 0.05	0.13 ± 0.06	0.09 ± 0.00
	DAG 36:4	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.12 ± 0.01
168 h	TAG 48:0	0.31 ± 0.06	0.24 ± 0.04	0.31 ± 0.06	0.26 ± 0.01
	TAG 50:0	0.28 ± 0.05	0.30 ± 0.03	0.37 ± 0.02**	0.31 ± 0.04
	TAG 50:1	1.71 ± 0.29	1.74 ± 0.14	2.02 ± 0.19	1.61 ± 0.09

	TAG 52:1	2.01 ± 0.33	2.29 ± 0.29	2.74 ± 0.18**	2.29 ± 0.32
	TAG 52:2	4.48 ± 0.68	5.05 ± 0.69	5.57 ± 0.30*	4.68 ± 0.42
	TAG 52:3	6.27 ± 0.70	7.02 ± 0.76	7.23 ± 0.26	6.74 ± 0.88
	TAG 52:4	5.34 ± 0.45	5.56 ± 0.51	6.92 ± 0.26**	5.69 ± 0.80
	TAG 54:0	0.13 ± 0.04	0.11 ± 0.06	0.09 ± 0.01	0.10 ± 0.01
	TAG 54:1	0.90 ± 0.16	1.16 ± 0.11	1.20 ± 0.08*	1.03 ± 0.15
	TAG 54:2	2.43 ± 0.51	3.05 ± 0.48	3.41 ± 0.30*	2.85 ± 0.45
	TAG 54:3	4.66 ± 0.60	5.40 ± 0.60	5.71 ± 0.41*	5.40 ± 0.65
	TAG 54:4	6.77 ± 0.32	7.80 ± 0.74	7.73 ± 0.40	7.99 ± 0.96*
	TAG 54:6	6.25 ± 0.48	6.38 ± 0.56	8.26 ± 0.54*	6.95 ± 1.04
	DAG 32:0	10.27 ± 0.61	9.10 ± 0.76*	9.79 ± 0.42	10.00 ± 0.45
	DAG 34:0	0.20 ± 0.03	0.22 ± 0.04	0.21 ± 0.03	0.19 ± 0.01
	DAG 34:1	2.23 ± 0.18	2.19 ± 0.32	2.32 ± 0.34	2.67 ± 0.06
	DAG 34:2	0.52 ± 0.08	0.70 ± 0.10*	0.69 ± 0.08	0.65 ± 0.04
	DAG 36	0.51 ± 0.07	0.35 ± 0.05**	0.48 ± 0.07	0.46 ± 0.04
	DAG 36:1	4.72 ± 0.60	3.54 ± 0.49**	3.75 ± 0.25**	4.15 ± 0.17
	DAG 36:2	1.15 ± 0.09	1.37 ± 0.34	1.11 ± 0.07	1.12 ± 0.02
	DAG 36:3	0.08 ± 0.03	0.06 ± 0.01	0.06 ± 0.04	0.06 ± 0.02
	DAG 36:4	0.17 ± 0.01	0.15 ± 0.03	0.18 ± 0.02	0.14 ± 0.04

Data are expressed as means ± SD; all samples were prepared in triplicate, and the experiments were repeated twice; n = 6. Results with a normal distribution were tested using one-way analysis of variance, whereas non-parametric Kruskal–Wallis test was used to analyze the results that were not normally distributed; significance: ** —$p < 0.01$, * — $p < 0.05$.

Oświadczenia współautorów o udziale w publikacjach

Oświadczenie współautorów o udziale w publikacji

Litwin A., Nowak, M., Różalska, S. 2020. Entomopathogenic fungi: unconventional applications. *Reviews in Environmental Science and Bio/Technology*, vol. 19, 23-42. https://doi.org/10.1007/s11157-020-09525-1

Imię i nazwisko	Szacunkowy udział [%]	Opis działań
mgr Anna Litwin	65	• Współudział w przygotowaniu koncepcji pracy. • Przygotowanie tekstu manuskryptu. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. .....Anna Litwin..... (podpis współautora)
mgr Monika Nowak	5	• Przygotowanie rozdziału dotyczącego metabolitów wtórnych grzybów entomopatogennych. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. ....Monika Nowak.... (podpis współautora)
dr hab. Sylwia Różalska, prof. UŁ	30	• Autor korespondujący. • Współudział w przygotowaniu koncepcji pracy. • Nadzór nad postępami prac. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. .....Sylwia Różalska..... (podpis współautora)

Oświadczenie współautorów o udziale w publikacji

Litwin A., Bernat P., Nowak M., Słaba M., Różalska S. 2021. Lipidomic response of the entomopathogenic fungus *Beauveria bassiana* to pyrethroids. *Scientific Reports*, vol. 11, 21319.
https://doi.org/10.1038/s41598-021-00702-y

Imię i nazwisko	Szacunkowy udział [%]	Opis działań
mgr Anna Litwin	65	• Udział w realizacji badań: ○ przygotowanie prób do oznaczenia profilu fosfolipidowego, oznaczenia zawartości ergosterolu, lipidów neutralnych i oosporeiny; ○ przeprowadzenie mineralizacji w celu określenia zawartości metali w biomasie *B. bassiana*; ○ wykonanie ekstrakcji i oznaczenia zawartości pyretroidów w biomasie *B. bassiana* i w pożywce pohodowlanej przy użyciu chromatografii gazowej sprzężonej ze spektrometrem mas (GC-MS); ○ określenie wpływu pyretroidów na wzrost, aktywność metaboliczną, przepuszczalność błon komórkowych oraz występowanie wolnych rodników w komórkach badanego grzyba. • Interpretacja uzyskanych wyników. • Przeprowadzenie analiz statystycznych. • Przygotowanie manuskryptu obejmujące opracowanie graficzne wyników, opis wstępu, materiałów i metod, uzyskanych wyników. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. .....Anna Litwin..... (podpis współautora)
dr hab. Przemysław Bernat, prof. UŁ	10	• Oznaczenie fosfolipidów grzybowych (z wykorzystaniem techniki LC-MS/MS); współudział w interpretacji uzyskanych wyników oraz nadzór nad przygotowaniem ich opisu. • Oznaczanie zawartości ergosterolu (metodą GC-MS) i lipidów neutralnych (metodą LC-MS/MS); współudział w interpretacji uzyskanych wyników oraz nadzór nad przygotowaniem ich opisu. ..... (podpis współautora)

mgr Monika Nowak	5	• Analiza ilościowa oosporeiny (metodą LC-MS/MS); współudział w interpretacji uzyskanych wyników oraz w przygotowaniu ich opisu. .....Monika Nowak..... (podpis współautora)
dr hab. Mirosława Słaba, prof. UŁ	5	• Oznaczanie zawartości metali (metodą absorpcyjnej spektrometrii atomowej - AAS); współudział w interpretacji uzyskanych wyników oraz nadzór nad przygotowaniem ich opisu. ..... (podpis współautora)
dr hab. Sylwia Różalska, prof. UŁ	15	• Autor korespondujący. • Współudział w przygotowaniu koncepcji pracy oraz w planowaniu badań. • Nadzór nad postępami pracy badawczej. • Udział w realizacji badań: o oznaczenie wolnych rodników; o przeprowadzenie analiz mikroskopowych. • Współudział w interpretacji uzyskanych wyników. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. .....Sylwia Różalska..... (podpis współautora)

Oświadczenie współautorów o udziale w publikacji

Litwin A., Mironenka J., Bernat P., Soboń A., Różalska S. 2023. Accumulation of pyrethroids induces changes in metabolism of the entomopathogenic fungus *Beauveria bassiana*—Proteomic and lipidomic background. *Ecotoxicology and Environmental Safety*, vol. 249, 114418.
https://doi.org/10.1016/j.ecoenv.2022.114418

Imię i nazwisko	Szacunkowy udział [%]	Opis działań
mgr Anna Litwin	60	• Współudział w przygotowaniu koncepcji pracy. • Realizacja badań mających na celu określenie zdolności pyretroidów do akumulacji w grzybni *B. bassiana*. • Przeprowadzenie badań mających na celu określenie wpływu pyretroidów na: ○ proteom zewnątrz- oraz wewnątrzkomórkowy *B. bassiana*; ○ aktywność enzymów litycznych i lakazy; ○ profil acyloglicerolі. • Oznaczenie poziomu markerów stresu oksydacyjnego u grzyba entomopatogennego *B. bassiana* podczas akumulacji pyretroidów. • Interpretacja uzyskanych wyników. • Przeprowadzenie analiz statystycznych. • Przygotowanie manuskryptu obejmujące opracowanie graficzne wyników, opis wstępu, materiałów i metod, wyników i dyskusji. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. .....Anna Litwin..... (podpis współautora)
dr Julia Mironenka	5	• Współudział w interpretacji analiz proteomicznych. Julia Mironenka ..... (podpis współautora)
dr hab. Przemysław Bernat, prof. UŁ	10	• Udział w realizacji badań dotyczących wpływu pyretroidów na syntezę lipidów neutralnych: oznaczenie profilu acyloglicerolі, współudział w interpretacji uzyskanych wyników oraz nadzór nad przygotowaniem ich opisu. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. ..... (podpis współautora)

dr Adrian Soboń	5	• Współudział w optymalizacji metody ekstrakcji białek do badań proteomicznych. Soboń …….. (podpis współautora)
dr hab. Sylwia Różalska, prof. UŁ	20	• Autor korespondujący. • Współudział w przygotowaniu koncepcji pracy oraz w planowaniu badań. • Nadzór nad postępami pracy badawczej. • Współudział w realizacji badań mających na celu określenie wpływu zakumulowanych pyretroidów na powstawanie stresu oksydacyjnego u grzyba entomopatogennego *B. bassiana*. • Współudział oraz nadzór nad przygotowaniem tekstu manuskryptu. • Współudział w przygotowaniu odpowiedzi na uwagi recenzentów. Sylwia Różalska …….. (podpis współautora)