

47. Mycotoxin Workshop

01–03 June 2026, Berlin



Society for Mycotoxin Research
Gesellschaft für Mykotoxinforschung e.V.

Preface

Dear Participants,

We welcome you to the 47. Mycotoxin Workshop 2026 in Berlin, organised by the Society for Mycotoxin Research in cooperation with the German Federal Institute for Risk Assessment (BfR). For decades, the BfR (and its predecessor institutes) has been dedicated to the analysis and assessment of mycotoxins in food and feed and hosts the National Reference Laboratory for Mycotoxins and Plant Toxins. We are therefore delighted that the Mycotoxin Workshop is taking place for the third time in Berlin—after 2002 and 2016—at the BfR's facilities. We can look forward to 42 lectures and nearly 100 poster presentations during the 2.5 days. For the first time, the scientific programme includes poster pitches to make the poster sessions more engaging.

This year we welcome more than 200 participants from 25 countries and extend a warm greeting to all our colleagues—including those who have travelled long distances to join us. Characterised by international diversity and a spirit of open debate, the Mycotoxin Workshop seeks to reduce hierarchies and promote direct networking among scientists worldwide. The broad range of topics has always offered participants a valuable opportunity to broaden their horizons and look beyond their own fields of research. It is also a place to reunite with long-time colleagues and friends. The accompanying social programme supports these connections while offering a glimpse of Berlin's many facets.

Each Mycotoxin Workshop is organised on a voluntary, non-profit basis by local organisers and the Society for Mycotoxin Research. The Society remains committed to keeping registration fees as low as possible and aims to give young scientists in particular the opportunity to participate and present their research. The Society relies on the support of its members and on the commitment of dedicated individuals who have supported the mycotoxin community for decades. New members are always welcome.

We hope that everyone will enjoy a fruitful exchange of ideas and scientific insights, engaging scientific discussions, and a wonderful time in Berlin.

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German Federal Institute for Risk Assessment

Dr Benedikt Cramer
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Contents

Preface	2
Contents	4
1 Programme	11
2 Lecture Abstracts	19
2.1 L1 - A rapid in-solution colorimetric aptamer-based assay for the detection of fumonisins in maize	19
2.2 L2 - Development and validation of a UHPLC-HRMS method for epimer-specific quantification of ergot alkaloids for analysis of Canadian wheat	21
2.3 L3 - Tracing the metabolic fate of ochratoxin a in maize using stable isotope-assisted LC-HRMS	22
2.4 L4 - CCS trendlines in non-targeted analysis: application to <i>Fusarium proliferatum</i> secondary metabolism	24
2.5 L5 - Mycotoxins in Ethiopian Food Systems: Current Burden and Emerging Biotechnology as a Solution	26
2.6 L6 - Mycotoxins and plant toxins in plant-based drinks – Survey of the German market 2024-2025	27
2.7 L7 - Fungi and Mycotoxins Contamination of Selected Aromatic Plants	28
2.8 L8 - Seasonal mycotoxin exposure and risks in Swiss children based on urinary biomonitoring	29
2.9 L9 - Mycotoxin Profiles of <i>Alternaria</i> spp. in Oat Grains under Different Water Activity and Temperature Conditions	31
2.10 L10 - Skin microbiota of processing tomato fruits in relation to <i>Alternaria</i> black spot and mycotoxin contamination	33
2.11 L11 - Growth Modelling of T-2 and HT-2 Toxin-Producing <i>Fusarium</i> spp. in Oat-Based Medium	35
2.12 L12 - Differential responses of <i>Fusarium avenaceum</i> strains to oxidative stress: impacts on growth and mycotoxin yield.	37
2.13 L13 - Mycotoxin threats in a changing climate: effects of interacting abiotic factors on fungal growth and OTA/CIT production in rice	39
2.14 L14 - New PKS and metabolites putatively involved in ochratoxin A accumulation by <i>Penicillium nordicum</i> in NaCl-rich environments	41
2.15 L15 - Quantifying interaction dynamics among mycotoxigenic fungi	42
2.16 L16 - Genetic characterization and geographical variation in mycotoxin profiles of <i>Fusarium culmorum</i> from wheat in South of Italy	44
2.17 L17 - Detection of Secondary Metabolite Biosynthetic Genes Across the <i>Fusarium oxysporum</i> Species Complex	45
2.18 L18 - Shuffling of the patulin biosynthetic gene cluster in <i>Penicillium roqueforti</i>	46

2.19 L19 - Population genomics and aflatoxin production of <i>Aspergillus flavus</i> and <i>Aspergillus oryzae</i> from China	47
2.20 L20 - Pathogenicity-Associated Metabolic Differentiation in <i>Trichoderma afroharzianum</i>	48
2.21 L21 - Going with the wind...and other weather variables to predict mycotoxin risk.	50
2.22 L22 - An Integrated AI-Based Text-Mining Pipeline for Automated Extraction of Mycotoxin Occurrence and Analytical Data from Scientific Literature	52
2.23 L23 - A novel approach for predicting aflatoxin B ₁ production using regression models and whole-cell biosensors in moldy maize and peanut kernels	54
2.24 L24 - Untargeted HRMS screening of maize: a QC-Based Workflow with Linear and Non-Linear Data Characterisation	56
2.25 L25 - NAMs for Food Safety: <i>In Silico</i> Modelling of Bacterial Membranes to Explore Mycotoxin Bioavailability in the Intestinal Compartment	57
2.26 L26 - Novel insights into the toxicological profile of <i>Alternaria infectoria</i> mycotoxins	59
2.27 L27 - Evaluating the Genotoxic Potential of Emerging Mycotoxins Using Stepwise Approach	61
2.28 L28 - Early oxidative and mitochondrial stress responses to tenuazonic acid in human esophageal cells	63
2.29 L29 - In vitro digestion of lipophilic mycotoxins enniatins and beauvercin	65
2.30 L30 - Enniatins and Their Mixture Induce Cytotoxicity in Rainbow Trout (SOB-15) Hepatocytes Through Early Disruption of Heme Biosynthesis	66
2.31 L31 - Heme biosynthesis under mycotoxin pressure in Atlantic salmon: a multi-target investigation	68
2.32 L32 - How the mycotoxin deoxynivalenol exacerbates the genotoxicity of heme iron	70
2.33 L33 - Deoxynivalenol disrupts the blood-testis barrier in prepubertal and adult mice: a possible cytokine-mediated effect	72
2.34 L34 - Shaping the immunomodulation in colon: how high glucose conditions modulate the immunomodulatory properties of mycotoxins	74
2.35 L35 - Unraveling the toxicokinetics of T-2 and HT-2 toxin in humans and developing a physiologically-based kinetic model	75
2.36 L36 - Beyond aflatoxin B ₁ : Mutagenicity assessment and evaluation of topoisomerase-poisoning potential of selected aflatoxin B ₁ precursors	76
2.37 L37 - Acute Toxic Effects of <i>Stachybotrys chartarum</i> Bioaerosols	78
2.38 L38 - Hydrothermal treatment with sodium metabisulfite of deoxynivalenol contaminated maize as effective tool of inactivation	79
2.39 L39 - Characterization of a detoxified deoxynivalenol metabolite supporting microbial mitigation strategies in food safety	81
2.40 L40 - Impact of UV-C treatment on <i>Alternaria</i> spp. growth and <i>Alternaria</i> mycotoxins <i>in vitro</i> and in tomato	83

2.41 L41 - From the Plate to the Plant: Discovering the biocontrol potential of <i>Hanseniaspora uvarum</i> against <i>Aspergillus flavus</i>	84
2.42 L42 - Transcriptomic profiling of aflatoxin B1 exposed and medicinal herb supplemented pig liver	86
3 Poster Abstracts	88
3.1 P1 - Species-level <i>Fusarium</i> resolution in cereals: a TEF1 metataxonomic approach	88
3.2 P2 - Exploring Toxic Interactions Between Aflatoxin B1 and Its Precursors	90
3.3 P3 - Long-Term Monitoring of Mycotoxin Occurrence in Czech Malting Barley under Climate Variability	91
3.4 P4 - Hybrid in vitro/in silico approach to elucidate the effect of mycoestrogens on barrier integrity via tight junction protein claudin-4	92
3.5 P5 - Impact of maize–bean intercropping on crop growth and <i>Fusarium</i> mycotoxin contamination	94
3.6 P6 - Sulfation as a detoxifying mechanism for the estrogenicity of the mycotoxin alternariol	95
3.7 P7 - Reduction of polar mycotoxins in malting steeping water using the ACMalt recycling technology	97
3.8 P8 - Mycotoxin analysis using strip tests and chromatographic methods	99
3.9 P9 - Targeting Tenuazonic Acid: Development of a Multiplex Suspension Array Fluorescence Immunoassay for <i>Alternaria</i> and Major Regulated Mycotoxins	100
3.10 P10 - Tetramic acid derivatives from <i>Alternaria</i> and <i>Fusarium</i> counteract zearalenone-induced estrogenic signaling in 2D Ishikawa and 3D MCF-7 cells	102
3.11 P11 - Target fishing in the "kinome": integrated in silico/in vitro discovery of novel kinase inhibitory activities of alternariol	104
3.12 P12 - Interinstitutional Network for Biomonitoring Citrinin Exposure in Children and Adolescents: Fostering Interdisciplinary Collaboration Between Portugal and Germany	105
3.13 P13 - Atranone – an overlooked secondary metabolite?	106
3.14 P14 - Multi-country survey of major mycotoxins in maize, sorghum and millet across Africa within the UP-RISE framework	108
3.15 P15 - Exploring mycotoxin interactions with heme transporters in Atlantic salmon: a computational journey	110
3.16 P16 - <i>In Vitro</i> Binding Evaluation of Silicoglycidol, a Mycotoxin Binding Technology Under Simulated Gastrointestinal Conditions of Broiler Chickens	111
3.17 P17 - Simulating the Upper and Lower Gastrointestinal Tract of a Cereal-Based Sample: An In Vitro Study on modified HT-2 and T-2 Toxins	112
3.18 P18 - Volatile apocarotenoids treatment in management of <i>Fusarium oxysporum</i> f. sp. <i>lini</i> infection	114
3.19 P19 - Degradation of mycotoxin AFB ₁ using bacterial isolate <i>Rhodococcus</i> sp. (SFFA/2)	116

3.20 P20 - LC-MS/MS analysis of aflatoxin B ₁ and fumonisin B ₁ in maize and black soldier fly larvae	117
3.21 P21 - Mycotoxins and plant toxins in plant protein concentrates used for meat alternatives in the German market	119
3.22 P22 - Addressing Multi-Mycotoxin Analysis in Complex Food Matrices: Validation of a Multiplex Flow Cytometric Immunoassay	121
3.23 P23 - High-Throughput Profiling of Mycotoxigenic Fungi in Spanish Cereal Soils	123
3.24 P24 - Analysis of 10 mycotoxins in different agricultural and food/feed matrices using LC-MS/MS	125
3.25 P25 - Metataxonomic profiling of <i>Tenebrio molitor</i> gut microbiota after exposure to aflatoxin B ₁ , fumonisin B ₁ , and deoxynivalenol	126
3.26 P26 - Molecular Determinants of Functional Tenuazonic Acid Immunoassays: Sequence–Structure Correlates of Competitive Performance	128
3.27 P27 - Rhizobacterial VOCs and their potential to counteract mycotoxigenic fungi	130
3.28 P28 - Determination of ochratoxin A in pistachio and cheese: the inter-laboratory validation study	131
3.29 P29 - Mycotoxins modulate oxaliplatin-induced phagocytosis of colon cancer cells via oxidative stress	133
3.30 P30 - Analysis of sterigmatocystin in a controlled carry-over study in dairy cows	134
3.31 P31 - Elucidating the effect of deoxynivalenol on the activity profile of topoisomerase inhibitor SN-38	136
3.32 P32 - Mycotoxin Profile of Maize in AP Vojvodina, Serbia and the Republic of Srpska, BiH: Influence of 2024 Weather Conditions	138
3.33 P33 - Mycotoxin exposure following an eight-week vegan or meat-rich dietary intervention: A randomized-controlled trial in healthy individuals	140
3.34 P34 - Apocarotenoids and fusaric acid: potential metabolic cross-talk during flax - <i>Fusarium</i> interaction	142
3.35 P35 - Sharper Eyes on Ergot: Advancing Detection in Wheat	144
3.36 P36 - Comparison of ELISA, UHPLC-MS/MS, and Development of UHPLC-HRMS Method for Ergot Alkaloid Quantification in Wheat	145
3.37 P37 - Aflatoxins in maize and milk in Serbia: Multi-year trends and implications for food and feed safety	146
3.38 P38 - Zearalenone modulates the expression of apoptotic markers in ovarian cancer cells	148
3.39 P39 - The role of flavonoids in maize (<i>Zea mays</i> L.) fungal resistance	149
3.40 P40 - Stability of regulated and emerging mycotoxins in water slurry mixtures of corn and peanut matrices under different storage conditions	150
3.41 P41 - Hepatic metabolism of naturally occurring ergot alkaloids: Insights from human and porcine liver microsomes	152

3.42 P42 - The effect of sterigmatocystin on the glutathione redox system and lipid peroxidation in broiler chicken with selenium supplementation	153
3.43 P43 - The effect of acerola and wild rose extracts on mould growth and mycotoxin production	154
3.44 P44 - Comparative lung toxicity of sterigmatocystin and its precursors	156
3.45 P45 - Correlations Between Maize Chemical Composition and <i>Fusarium</i> Mycotoxin Occurrence	158
3.46 P46 - Disruption of heme biosynthesis as a novel mode of action of enniatins and beauvericin	160
3.47 P47 - Fungal Infection and Mycotoxin Profile in Spanish Oat Crops: Impact of Meteorological Conditions on <i>Fusarium</i> Prevalence	161
3.48 P48 - Consumer Awareness of Mycotoxin Contamination in Food	163
3.49 P49 - When mycotoxins taste bitter (and beyond): AI-driven discovery of bitter receptors-fungal indolizidine alkaloids interaction	164
3.50 P50 - Moldy foods: how to predict mycotoxin production and migration in foods?	165
3.51 P51 - Effect of Non-Thermal Plasma Exposure on the Mycotoxin Profile of <i>Aspergillus niger</i>	167
3.52 P52 - Developing a new methodology for the detection of mycotoxins in human breast milk	168
3.53 P53 - Phenolic Compounds in the Control of <i>Fusarium</i> Growth and Toxigenicity	169
3.54 P54 - Zero-Waste Valorization of Pomegranate Peel for the Control of <i>Aspergillus flavus</i> Growth and Aflatoxin B1 Production	170
3.55 P55 - Mycotoxin Contamination in Peanuts (<i>Arachis hypogaea L.</i>) at Post-harvest Stage in Eastern Ethiopia	171
3.56 P56 - Isolation and structure elucidation of biologically active secondary metabolites from <i>Stachybotrys</i> spp	172
3.57 P57 - Contribution of terrestrial processes in reducing environmental Deoxynivalenol levels	174
3.58 P58 - Hidden Estrogenic Burden in Finished Feed: Co-Occurrence of Phytoestrogens and Mycoestrogens in European Livestock Diets	176
3.59 P59 - From <i>Alternaria</i> extract to alterperyleneol: Discovery of an immunosuppressive mycotoxin targeting NF-κB	178
3.60 P60 - Ergot Alkaloids in Italian Cereals and Cereal-Based Products: A Five-Year Monitoring Study (2020–2025)	180
3.61 P61 - Comparative Intestinal Permeability and Molecular Responses to Major Aquafeed Mycotoxins in RTgutGC Cells	182
3.62 P62 - Long-term monitoring of mycotoxins in barley, malt and beer: trends, transfer and analytical challenges	184
3.63 P63 - Organic vs. Conventional and Jars vs. Pouches: A Comparison of Patulin incidence in Apple-based Baby Food	185

3.64 P64 - Zearalenone and ochratoxin A induced hormonal imbalance in female reproduction	186
3.65 P65 - Airborne Aspergillus species in a Zoological Garden: diversity, cytotoxic effects and mycotoxin production	188
3.66 P66 - Combined estrogenic effects of zearalenone with other myco-, xeno-, and phytoestrogens on <i>Tg(vtg1:mCherry)</i> zebrafish embryos	189
3.67 P67 - Potential of cold atmospheric plasma for mitigation of mycotoxin contamination in milk thistle	191
3.68 P68 - Ochratoxin A Quantification in Dry-Cured Ham: results from a preliminary comparison in different laboratories	193
3.69 P69 - Moldy foods: how to predict mycotoxin production and migration in foods?	194
3.70 P70 - Screening for Aflatoxins in Soybeans and Soybean-Derived Products Available on the German Market by Lateral Flow Assay	195
3.71 P71 - <i>In silico</i> discovery of potential dehydrogenases for Deoxynivalenol biodegradation	196
3.72 P72 - Synergistic Effects of Fumonisin B1 and Polystyrene Microplastics on Porcine Renal and Ovarian Explants	198
3.73 P73 - Occurrence of mycotoxins in cheese with special cheese toppings	200
3.74 P74 - Mycotoxin Monitoring in the Context of Climate Change	201
3.75 P75 - Analysis of mycotoxins and cortisol levels in tissues of wild animals	203
3.76 P76 - Survey for mycotoxins and plant toxins contamination in medical foods	205
3.77 P77 - Balancing Matrix, Mesh and Metrology: Cross-Platform Performance of Naturally Contaminated Mycotoxin Reference Materials	207
3.78 P78 - Validation of an LC–MS/MS Multi-Mycotoxin Method in Plant-Based Meat Alternatives: Brand-Level Assessment Across Soy, Wheat and Seitan Matrices	209
3.79 P79 - Rising Risks in Europe: Key Findings from the 2025 Mycotoxin Survey	210
3.80 P80 - The presence of moulds and mycotoxins in animal feed collected between 2024 and 2025	211
3.81 P81 - Zearalenone and α -zearalenol Modulate Cell Cycle Progression and Affect NRF2 Signaling in an Inflammation-Based Model of Breast Cancer Cells	213
3.82 P82 - The Interaction of a Blend of Non-Starch Polysaccharide Enzymes with Deoxynivalenol, and its Modified Form Deoxynivalonol-3-Glucoside, on the Growth Performance and Toxicokinetic in Broiler Chickens	214
3.83 P83 - Common mycotoxins in dry dog and cat feed	215
3.84 P84 - To bind or to degrade? Fate of deoxynivalenol and ochratoxin A during soy fermentation	216
3.85 P85 - Messy matrix, clean results: optimising sample clean-up for ochratoxin A and deoxynivalenol detection in fermented soy	218

3.86 P86 - Occurrence of T-2 and HT-2 mycotoxins in different spring barley varieties and concentration changes during the malting process	220
3.87 P87 - Development and validation of a LC-MS/MS multi-method for the determination of mycotoxins in insect-based novel food	222
3.88 P88 - Development and Evaluation of a Lateral Flow Immunoassay for Tenuazonic acid	224
3.89 P89 - <i>Fusarium</i> infection and mycotoxin production in <i>Cannabis sativa</i> : Implications for crop safety and consumer health	225
3.90 P90 - Occurrence of T-2, HT-2 and Their Glucosides in Apple-Based Beverages	226
3.91 P91 - <i>Metarhizium</i> spp. encode an ochratoxin cluster and a high efficiency ochratoxin-degrading amidohydrolase	228
3.92 P92 - A glutathione-S-transferase from <i>Fusarium graminearum</i> inactivating trichothecenes by epoxide opening: a role in self-protection or fungus-fungus interaction?	229
3.93 P93 - ThermELUTE Light – Evaluation of an accelerated sample preparation for quantification of aflatoxins and ochratoxin A by SMART cartridges	230
4 Index of Authors	231

1 Programme

Sunday, 31 May 2026

Get together

@Nikolaiviertel, 10178 Berlin

18:30 **Meeting in front of St. Nicolas Church (Nikolaikirche)**
<https://maps.app.goo.gl/sukPhFwqnuiWW7uF9>

Monday, 01 June 2026

Registration and Welcome

08:00 **Registration (Forum)**
 Bundesinstitut für Risikobewertung (BfR), location Marienfelde
 Diedersdorfer Weg 1
 12277 Berlin
<https://maps.app.goo.gl/H2NYEKdQbbvaCqgc9>

09:00–09:15 **Welcome session**

Session I: Analytics

Session Chairs: Michael Sulyok (BOKU University, Austria) and Benedikt Cramer (University of Münster, Germany)

09:15–09:30 **L1 - A rapid in-solution colorimetric aptamer-based assay for the detection of fumonisins in maize**
 Vincenzo Lippolis₂, Institute of Sciences of Food Production, National Research Council of Italy, Bari, Italy

09:30–09:45 **L2 - Development and validation of a UHPLC-HRMS method for epimer-specific quantification of ergot alkaloids for analysis of Canadian wheat**
 Chamali Kodikara, Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Manitoba

09:45–10:00 **L3 - Tracing the metabolic fate of ochratoxin A in maize using stable isotope-assisted LC-HRMS**
 Filip Petronijevic, Institute of Bioanalytics and Agro-Metabolomics, Department of Agricultural Sciences, BOKU University, Tulln, Austria

10:00–10:15 **L4 - CCS trendlines in non-targeted analysis: application to *Fusarium proliferatum* secondary metabolism**
 Guillem Campmajó₂, Department of Food and Drug, University of Parma, Parma, Italy

Poster Pitch Session I

10:15–10:30

P35 - Sharper Eyes on Ergot: Advancing Detection in Wheat

Chamali Kodikara, Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Manitoba, Canada

P50 - A multidisciplinary framework for risk assessment of mycotoxins in moldy foods supporting consumer safety and food waste reduction

Monika Coton, University of Brest, INRAE, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Plouzané, France

P69 - Moldy foods: how to predict mycotoxin production and migration in foods?

Charlotte Réant, University of Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, Plouzané, France

P74 - Mycotoxin Monitoring in the Context of Climate Change

Astrid Schöberl, Feed and Food Quality, Safety & Innovation, Tulln, Austria

P3 - Long-Term Monitoring of Mycotoxin Occurrence in Czech Malting Barley under Climate Variability

Sylvie Běláková, Research Institute of Brewing and Malting, Czech Republic (Czechia)

P90 - Occurrence of T-2, HT-2 and Their Glucosides in Apple-Based Beverages

Annalisa Melanie Völsch, Institute of Food Chemistry, University of Münster, Münster, Germany

P56 - Isolation and structure elucidation of biologically active secondary metabolites from *Stachybotrys* spp.

Mehrsima Montaser Kouhsari, Institute of Food Chemistry, University of Münster, Münster, Germany

P13 - Atralone – an overlooked secondary metabolite?

Mareike Dabisch-Ruthe, OWL University of Applied Sciences and Arts, Department of Agriculture, Food and Health, Microbiology, Lemgo, Germany

10:30–11:30

Coffee break / Poster exhibition (uneven)

Location: Forum and rooms D145/146

Session II: Occurrence and management

Session Chairs: Sarah De Saeger (University of Ghent, Belgium) and Magdalena Twaruzek (University of Bydgoszcz, Poland)

11:30–11:45

L5 - Mycotoxins in Ethiopian Food Systems: Current Burden and Emerging Biotechnology as a Solution

Abdi Mohammed, Haramaya University, Ethiopia

11:45–12:00

L6 - Mycotoxins and plant toxins in plant-based drinks – Survey of the German market 2024-2025

Arnold Bahlmann, German Federal Institute for Risk Assessment, Berlin, Germany

12:00–12:15	<p>L7 - Fungal and Mycotoxins Contamination of Selected Aromatic Plants</p> <p>Marijana Sokolovic₂, Croatian Veterinary Institute, Poultry Centre, Feed Analysis Laboratory, Zagreb, Croatia</p>
12:15–12:30	<p>L8 - Seasonal mycotoxin exposure and risks in Swiss children based on urinary biomonitoring</p> <p>Lucienne Zinsstag, Swiss Tropical and Public Health Institute, Allschwil, Switzerland</p>
12:30–12:45	<p>L9 - Mycotoxin Profiles of <i>Alternaria</i> spp. in Oat Grains under Different Water Activity and Temperature Conditions</p> <p>Tiago Nazareth, University of Lleida, Lleida, Spain</p>
12:45–14:15	<p>Lunchbreak (and directly before: group photo)</p> <p>Location: Canteen</p>
<p>Session III: Biology, ecology and genetics I</p> <p>Session Chairs: Monika Coton (University of Brest, France) and Antonio Moretti (Research National Council, Italy)</p>	
14:15–14:30	<p>L10 - Skin microbiota of processing tomato fruits in relation to <i>Alternaria</i> black spot and mycotoxin contamination</p> <p>Léna Dole, Université de Montpellier, Montpellier, France</p>
14:30–14:45	<p>L11 - Growth Modelling of T-2 and HT-2 Toxin-Producing <i>Fusarium</i> spp. in Oat-Based Medium</p> <p>Jean Correia Costa₂, Applied Mycology Unit, Department of Food Technology, Engineering and Science, University of Lleida, Lleida, Spain</p>
14:45–15:00	<p>L12 - Differential responses of <i>Fusarium avenaceum</i> strains to oxidative stress: impacts on growth and mycotoxin yield</p> <p>Aurélie Touya, UR1264 Mycology and Food Safety (MycSA), INRAE, Villenave d'Ornon, France</p>
15:00–15:15	<p>L13 - Mycotoxin threats in a changing climate: effects of interacting abiotic factors on fungal growth and OTA/CIT production in rice</p> <p>Carolina Sousa Monteiro, LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal</p>
15:15–15:30	<p>L14 - New PKS and metabolites putatively involved in ochratoxin A accumulation by <i>Penicillium nordicum</i> in NaCl-rich environments</p> <p>Micaela Álvarez, Complutense University of Madrid, Spain</p>
15:30–15:45	<p>L15 - Quantifying interaction dynamics among mycotoxigenic fungi</p> <p>Darina Balková, Department of Sustainable Crop Production (DI.PRO.VE. S.), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy</p>
15:45–16:45	<p>Coffee break / Poster exhibition (even)</p> <p>Location: Forum and rooms D145/146</p>
16:30	<p>Visits of selected facilities of the BfR (upon reservation)</p> <p>Location: Meeting in groups inside/outside the conference building</p>

18:00 **Welcome drinks and networking**

Location: Forum and garden

18:30 **Barbecue @BfR garden**

Location: Forum and garden

Tuesday, 02 June 2026

Registration

08:00 **Registration (Forum)**

Session IV: Biology, ecology and genetics II

Session Chairs: Jessica Gil Serna (Complutense University of Madrid, Spain) and Jovana Kos (University of Novi Sad, Serbia)

09:00–09:15 **L16 - Genetic characterization and geographical variation in mycotoxin profiles of *Fusarium culmorum* from wheat in South of Italy**

Antonio Moretti, CNR-ISPA Research National Council of Italy, Institute of Sciences of Food Production, Bari, Italy;

09:15–09:30 **L17 - Detection of Secondary Metabolite Biosynthetic Genes Across the *Fusarium oxysporum* Species Complex**

Daria Carella, Sapienza Università di Roma, Italy; CNR-ISPA, Bari, Italy

09:30–09:45 **L18 - Shuffling of the patulin biosynthetic gene cluster in *Penicillium roqueforti***

Monika Coton, University of Brest, INRAE, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Plouzané, France

09:45–10:00 **L19 - Population genomics and aflatoxin production of *Aspergillus flavus* and *Aspergillus oryzae* from China**

Feng-Yan Bai, Institute of Microbiology, Chinese Academy of Sciences, Beijing, People's Republic of China

10:00–10:15 **L20 - Pathogenicity-Associated Metabolic Differentiation in *Trichoderma afroharzianum***

Annette Pfordt, Georg-August Universität Göttingen, Göttingen, Germany

Poster Pitch Session II

10:15–10:30

P84 - To bind or to degrade? Fate of deoxynivalenol and ochratoxin A during soy fermentation

Sita Venier, Toxicology of Contaminants Unit, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Fougères, France

P1 - Species-level Fusarium resolution in cereals: a TEF1 metataxonomic approach

Sergio Alfas-Segura, Universidad Complutense Madrid, Madrid, Spain

P49 - When mycotoxins taste bitter (and beyond): AI-driven discovery of bitter receptors-fungal indolizidine alkaloids interaction

Ilaria Magnaldi, Department of Food and Drug, University of Parma, Parma, Italy

P4 - Hybrid in vitro/in silico approach to elucidate the effect of mycoestrogens on barrier integrity via tight junction protein claudin-4

Janice Bergen, University of Vienna, Vienna, Austria

P41 - Hepatic metabolism of naturally occurring ergot alkaloids: Insights from human and porcine liver microsomes

Nina Kühnhenrich, University of Münster, Münster, Germany

P61 - Comparative Intestinal Permeability and Molecular Responses to Major Aquafeed Mycotoxins in RTgutGC Cells

Cheila Pereira, LAQV-REQUIMTE University of Porto, Porto, Portugal

P59 - From Alternaria extract to alterperyleneol: Discovery of an immunosuppressive mycotoxin targeting NF-κB

Vanessa Partsch, Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria

P6 - Sulfation as a detoxifying mechanism for the estrogenicity of the mycotoxin alternariol

Eszter Borsos, Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria

10:30–11:30

Coffee break / Poster exhibition (even)

Location: Forum and rooms D145/146

Session V: Data Science, Omics and AI

Session Chairs: Luca Dellafiara (University of Parma, Italy) and Stefan Weigel (German Federal Institute for Risk Assessment, Berlin, Germany)

11:30–11:45

L21 - Going with the wind... and other weather variables to predict mycotoxin risk

Michail Evgeniou, DSM-Firmenich, Biodata Analytics, Tulln, Austria

11:45–12:00

L22 - An Integrated AI-Based Text-Mining Pipeline for Automated Extraction of Mycotoxin Occurrence and Analytical Data from Scientific Literature

Louis-Marie Cobigo, French Agency for Food, Environmental and Occupational Health & Safety (Anses), Toxicology of Contaminants Unit, Fougères, France

12:00–12:15

L23 - A novel approach for predicting aflatoxin B1 production using regression models and whole-cell biosensors in moldy maize and peanut kernels

Fuguo Xing, Chinese Academy of Agricultural Sciences Institute of Food Science and Technology, Beijing, People's Republic of China

12:15–12:30 **L24 - Untargeted HRMS screening of maize: a QC-Based Workflow with Linear and Non-Linear Data Characterisation**
Xenia Pascari, Reference Centre for Food and Feed Analysis, German Federal Institute for Risk Assessment (BfR), Berlin, Germany

12:30–12:45 **L25 - NAMs for Food Safety: In Silico Modelling of Bacterial Membranes to Explore Mycotoxin Bioavailability in the Intestinal Compartment**
Nuša Matjašec, Department of Computational Biological Chemistry, University of Vienna, Vienna, Austria

12:45–14:15 **Lunchbreak**
Location: Canteen

Session VI: Toxicology I

Session Chair: Siska Croubels (University of Ghent, Belgium) and Imourana Alassane-Kpembé (University of Montreal, Canada)

14:15–14:30 **L26 - Novel insights into the toxicological profile of Alternaria infectoria mycotoxins**
Florian Call, Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria

14:30–14:45 **L27 - Evaluating the Genotoxic Potential of Emerging Mycotoxins Using Stepwise Approach**
Silvia Gascón-Corella, Department of Pharmaceutical Sciences, Research Group MITOX, School of Pharmacy and Nutrition, Universidad de Navarra, Pamplona, Spain

14:45–15:00 **L28 - Early oxidative and mitochondrial stress responses to tenuazonic acid in human esophageal cells**
Dino Grgic, Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria

15:00–15:15 **L29 - In vitro digestion of lipophilic mycotoxins enniatins and beauvercin**
Marcus Trentzsch, German Federal Institute for Risk Assessment (BfR), Department Reference Centre for Food and Feed Analysis, Berlin, Germany

15:15–15:30 **L30 - Enniatins and Their Mixture Induce Cytotoxicity in Rainbow Trout (SOB-15) Hepatocytes Through Early Disruption of Heme Biosynthesis**
Luis Monzón Atienza, Institute of Marine Research, Bergen, Norway

15:30–15:45 **L31 - Heme biosynthesis under mycotoxin pressure in Atlantic salmon: a multi-target investigation**
Lorenzo Pedroni, Department of Food and Drug, University of Parma, Parma, Italy

15:45–16:45 **Coffee break / Poster exhibition (uneven)**
Location: Forum and rooms D145/146

16:15–17:15	General Meeting of the Society for Mycotoxin Research – Guests Welcome Location: Conference Hall
17:30	Bus departure: Sightseeing bus tour to Conference Dinner venue Location: Meeting in front of the conference building
19:30–23:30	Conference dinner @Pirates Berlin Location: Pirates Berlin, Mühlenstr. 78-80, 10243 Berlin https://maps.app.goo.gl/LeqaFK2n2kEckDsi6

Wednesday, 03 June 2026

Registration

08:00 **Registration (Forum)**

Session VII: Toxicology II

Session Chair: Ariane Vettorazzi (University of Navarra, Spain) and Doris Marko (University of Vienna, Austria)

09:00–09:15	L32 - How the mycotoxin deoxynivalenol exacerbates the genotoxicity of heme iron Margaux Lalaurie, Toxalim, Université de Toulouse, INRAE, ENVT, EI-Purpan, Toulouse, France
09:15–09:30	L33 - Deoxynivalenol disrupts the blood-testis barrier in prepubertal and adult mice: a possible cytokine-mediated effect Ana Laura Paulino Leite Gomes, Laboratory of Animal Pathology, Department of Preventive Veterinary Medicine, State University of Londrina, Londrina, Brazil
09:30–09:45	L34 - Shaping the immunomodulation in colon: how high glucose conditions modulate the immunomodulatory properties of mycotoxins Karolina Kowalska, Medical University of Lodz, Department of Cell Culture and Genomic Analysis, Lodz, Poland
09:45–10:00	L35 - Unraveling the toxicokinetics of T-2 and HT-2 toxin in humans and developing a physiologically-based kinetic model Hannah P. McKeon, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
10:00–10:15	L36 - Beyond aflatoxin B1: Mutagenicity assessment and evaluation of topoisomerase-poisoning potential of selected aflatoxin B1 precursors Noah Ratzler, Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria

10:15–10:30 **L37 - Acute Toxic Effects of *Stachybotrys chartarum* Bioaerosols**
Stefanie Klar, Unit Bioaerosols, Federal Institute for Occupational Safety and Health, Berlin, Germany

10:30–11:15 **Coffee break / Poster exhibition**
Location: Forum and rooms D145/146

Session VIII: Remediation and mitigation

Session Chairs: Katherine Muñoz (RPTU University Kaiserslautern-Landau) and Gerhard Adam (BOKU University, Tulln, Austria)

11:15–11:30 **L38 - Hydrothermal treatment with sodium metabisulfite of deoxynivalenol contaminated maize as effective tool of inactivation**
Susanne Kersten, Institute for Animal Nutrition, Friedrich – Loeffler – Institute, Brunswick, Germany

11:30–11:45 **L39 - Characterization of a detoxified deoxynivalenol metabolite supporting microbial mitigation strategies in food safety**
Clémence Rives, INRAE, UMR1331, Toxalim, Research Centre in Food Toxicology, Toulouse, France

11:45–12:00 **L40 - Impact of UV-C treatment on *Alternaria* spp. growth and *Alternaria* mycotoxins *in vitro* and in tomato**
Maria Agustina Pavicich, Centre of Excellence in Mycotoxicology and Public Health, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

12:00–12:15 **L41 - From the Plate to the Plant: Discovering the biocontrol potential of *Hanseniaspora uvarum* against *Aspergillus flavus***
Clara Melguizo, Department of Genetics, Physiology and Microbiology, Faculty of Biological Sciences, Complutense University of Madrid, Madrid, Spain

12:15–12:30 **L42 - Transcriptomic profiling of aflatoxin B1 exposed and medicinal herb supplemented pig liver**
Avon Augustin Nalpadan, Forschungsinstitut für Nutztierbiologie (FBN), Dummerstorf, Germany

12:30–13:00 **Closing session with poster awards**

2 Lecture Abstracts

2.1 L1 - A rapid in-solution colorimetric aptamer-based assay for the detection of fumonisins in maize

Vincenzo Lippolis¹, Maria Varsalona¹, Salvatore Cervellieri², Tiziana Forleo¹, Maria C. DeRosa³, Annalisa De Girolamo¹

¹ Institute of Sciences of Food Production, National Research Council of Italy, Bari, Italy

² Institute of Food Sciences, National Research Council of Italy, URT-Bari, Bari, Italy

³ Carleton University, Ottawa, Ontario, Canada

Fumonisins are mycotoxins produced by *Fusarium* species that frequently contaminate maize. Among all identified fumonisins (FBs), fumonisin B1 (FB1) and fumonisin B2 (FB2) are the most toxic causing esophageal cancer in humans. Due to their toxicity and widespread occurrence the European Commission has established maximum limits for various food products, expressed as sum of FB1 and FB2.

While several analytical methods have been reported for FBs detection, conventional ones are costly and complex, creating a need for rapid and cost-effective alternatives. Aptamers, single-stranded nucleic acids selected via SELEX, offer a promising selective tool for developing rapid methods to detect fumonisins.

A rapid in-solution colorimetric assay was developed, based on the interaction between gold nanoparticles (AuNPs) and an aptamer specific for FB1 detection in buffer solution and in maize samples. AuNPs were employed due to their ability to change color from red to purple upon aggregation, triggered by the aptamer-target interaction. The visual shift, detectable spectrophotometrically (520-620 nm), enabled straightforward monitoring of FB1 presence. The effects of various parameters, such as aptamer and AuNP concentrations, salt levels, incubation time, and reagent volumes, were tested to optimize the color intensity and absorbance ratio. The optimized assay exhibited a cross-reactivity against FB2 of 85%. The assay was applied to the determination of FBs, expressed as sum FB1 and FB2. A linear detection range between 0.08-0.4 µg/mL FBs in buffer standard solution, with a repeatability of $\leq 11\%$, was observed. The developed aptamer assay was tested on a real maize sample spiked with increasing FBs concentration. Following methanol-water extraction, the extract was filtrated and 5-fold diluted with buffer prior to the AuNPs assay. Although the assay exhibited a matrix effect, it demonstrated a linear detection range of 2,000-10,000 µg/Kg FBs in maize with a relative standard deviation $\leq 6\%$. The test can be performed in solution within 30 minutes requiring no specialized skills. Mean FBs recoveries from spiked maize samples ranged from 93% to 119 %, with repeatability lower than 10%. The trueness of the assay was further assessed using two maize reference materials for FBs, demonstrating good accuracy and precision.

The proposed rapid assay is simple, rapid and sensitive, making it suitable for screening large quantities of samples and ensuring the safety of cereal products.

Financial support from the HOLiFOOD project (grant agreement 101059813 under the European Union's 441 Horizon Europe Program for Research and Innovation) is gratefully acknowledged.

2.2 L2 - Development and validation of a UHPLC-HRMS method for epimer-specific quantification of ergot alkaloids for analysis of Canadian wheat

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² Department of Food & Human Nutritional Sciences, University of Manitoba, Winnipeg, Canada

³ Richardson Centre for Food Technology and Research, University of Manitoba, Winnipeg, Canada

Introduction: Ergot contamination caused by *Claviceps purpurea* is a persistent and re-emerging challenge in cereal production worldwide, posing risks to food and feed safety, international trade, and regulatory compliance. Reliable quantification of ergot alkaloids (EAs) remains analytically demanding due to strong matrix effects, structural similarity, and interconversion between -ine and -inine epimers. This study aimed to develop and validate a high-resolution mass spectrometry method capable of accurate, epimer-specific EA quantification and to demonstrate its broader applicability using a comprehensive Canadian wheat dataset.

Methods: An Ultra-High-Pressure-Liquid-Chromatography High-Resolution-Mass-Spectrometry (UHPLC-HRMS) method was developed to achieve chromatographic separation of all twelve major EAs. Ionization and fragmentation were optimized by direct infusion and chromatographic testing. Method validation included characterization of limits of detection (LOD) and quantification (LOQ), recovery, intra- and inter-day precision, matrix effects, and extract stability. Accuracy and robustness were evaluated using eleven certified, proficiency, and in-house reference materials. The validated method was applied to 134 Canadian wheat samples collected between 2020 and 2024 across ten crop regions, representing multiple wheat classes (CWRS, CWAD, CESRW) and grade categories.

Results: Matrix-matched calibration curves showed excellent linearity for all analytes ($R^2=0.9976-0.9998$). LODs ranged from 0.1 to 0.8 $\mu\text{g}/\text{kg}$ and LOQs from 0.4 to 2 $\mu\text{g}/\text{kg}$. Mean recoveries across five fortification levels were generally within accepted analytical criteria, with intra- and inter-day precision typically <15% RSD. Matrix effects ranged from 95% to 162%, supporting the necessity of matrix-matched calibration. Analysis of reference materials showed close agreement with certified or assigned values, with no systematic over- or under-estimation ($p > 0.05$). Application to Canadian wheat samples demonstrated substantial variability in both total EA concentrations and alkaloid profiles across years, regions, classes, and grades, with total EAs ranging from 0 to 800 $\mu\text{g}/\text{kg}$ and statistically significant differences among selected groups (ANOVA, $p < 0.05$).

Significance: This validated UHPLC-HRMS method provides a sensitive, selective, and epimer-resolved platform suitable for global ergot alkaloid monitoring. While demonstrated using Canadian wheat, the analytical method is broadly transferable to international cereal matrices and supports surveillance programs, risk-based management strategies, and regulatory method development for ergot contamination worldwide.

2.3 L3 - Tracing the metabolic fate of ochratoxin a in maize using stable isotope-assisted LC-HRMS

Filip Petronijevic¹, Gerlinde Wiesenberger^{1,2}, Christoph Büschl¹, Gerhard Adam², Franz Berthiller¹

¹ Institute of Bioanalytics and Agro-Metabolomics, Department of Agricultural Sciences, BOKU University, Tulln, Austria

² Institute of Microbial Genetics, Department of Agricultural Sciences, BOKU University, Tulln, Austria

Ochratoxin A (OTA) is a toxic fungal metabolite commonly occurring in plant-derived food and feed. As a xenobiotic, OTA can undergo metabolic transformation in plants, potentially altering its toxicity. The European Food Safety Agency panel on Contaminants in the Food Chain stated in their latest opinion that “more data on occurrence and toxicity of modified OTA are needed”.¹ While several OTA metabolites have been reported^{2,3}, most studies are limited to cell cultures, and the extent of OTA metabolism in intact plants remains largely unknown. In particular, it is unclear which metabolites are truly formed in-planta and to what extent OTA is metabolized or bound within plant tissues.

To comprehensively investigate OTA metabolism in maize, a stable isotope-assisted metabolomics approach was applied. Uniformly ¹³C-labelled OTA was produced and administered to mini-maize plants together with native OTA in a 1:1 ratio, enabling selective tracking of OTA-derived compounds and differentiation from endogenous plant metabolites. Plants were treated with 2 mg OTA per plant during cob development at two time points, 10 and 20 days after pollination. When applied at 20 days after pollination, recovery of parent OTA was close to the applied dose. In contrast, when applied at 10 days after pollination, only approximately 12% of the applied OTA was recovered, which could indicate extensive metabolic transformation and/or binding within plant tissues.

Plant extracts were analyzed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Isotope-assisted data processing using MetExtract II enabled selective extraction of OTA-derived signals based on co-eluting ¹²C/¹³C isotopologue pairs⁴. Annotation based on accurate mass measurements revealed approximately 70 putative OTA metabolites. While several metabolites corresponded to compounds previously described in the literature, numerous additional putative metabolites were detected, substantially expanding the known spectrum of OTA-derived compounds in plants. To further support structural characterization, high-resolution MS/MS spectra were acquired for the most abundant metabolites. Comparison of labelled and unlabelled fragment ions provided structural information and confirmed their origin from OTA. These results demonstrate that OTA undergoes extensive metabolism in maize, resulting in a diverse range of modified forms formed directly in planta.

This study reveals a greater extent of OTA metabolism in maize than previously recognized and confirms the in-planta formation of numerous OTA-derived metabolites. The applied stable isotope-assisted LC-HRMS approach enables comprehensive investigation of mycotoxin metabolism and contributes to improved understanding of modified OTA forms relevant for food safety and risk assessment.

References

- 1) EFSA CONTAM Panel, 2020. *EFSA Journal* 18(5): 6113.
- 2) Cramer, B. et al., 2008. *Journal of Agricultural and Food Chemistry* 56(14): 5673-5681.
- 3) Ruhland, M. et al., 1996. *Natural Toxins* 4: 254-260.
- 4) Bueschl, C. et al., 2017. *Analytical Chemistry* 89(17): 9518-9526.

2.4 L4 - CCS trendlines in non-targeted analysis: application to *Fusarium proliferatum* secondary metabolism

Guillem Campmajó¹, Irene Picicci¹, Antonia Susca², Antonio Moretti², Chiara Dall'Asta¹

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Ion mobility spectrometry (IMS) hyphenation to mass spectrometry (MS), often combined with liquid chromatography (LC), provides an additional separation dimension for resolving isobaric compounds. IMS not only increases method selectivity and sensitivity but also enhances confidence in metabolite identification by providing a robust chemical descriptor, the collision cross section (CCS), which is fundamentally related to the size, shape, and conformation of an ion [1]. Despite the availability of several databases and CCS prediction tools, there is still very limited data, especially with regard to fungal metabolites. Nevertheless, in recent years, CCS vs. m/z trendlines — typically observed for compounds belonging to the same chemical family — have gained interest in non-targeted analysis as a potential prioritisation strategy [2]. In this context, the present study aimed to exploit the potential of CCS trendlines in the non-targeted metabolomic analysis of *F. proliferatum* secondary metabolism. Thus, after a biphasic solid-liquid extraction, the polar fraction was analysed by liquid chromatography–traveling wave ion mobility spectrometry–high-resolution mass spectrometry (LC–TWIMS–HRMS).

Firstly, 13 *F. proliferatum* strains inoculated on autoclaved rice and exhibiting diverse fumonisins B (FBs) production were analysed by LC–TWIMS–HRMS, and experimental TWCCSN2 values were obtained for 111 secondary metabolites. In total, 272 ions were detected in positive ionisation mode (i.e., protonated, sodium, potassium, and ammonium adducts) and 110 in negative ionisation mode (i.e., deprotonated, chloride, and acetate adducts). All measurements showed repeatability with relative standard deviation (RSD) below 2%. As an additional step beyond system calibration, the acquired TWCCSN2 values were validated by comparison with previously reported data, when available, yielding good CCS deviations (ΔCCS , %). Secondly, TWCCSN2 vs. m/z plots were constructed for each ion, revealing four distinct CCS trendlines corresponding to fumonisin-related compounds (including fumonisins, their modified forms, and pathway intermediates), acyl fumonisins, beauvericins, and bikaverins. The fumonisin CCS trendline was subsequently compared with trendlines derived from i) previously reported experimental data and ii) CCS prediction tools, showing satisfactory agreement. Finally, the applicability of CCS trendlines as a prioritisation strategy in non-targeted analysis was evaluated for the detection and identification of fumonisin-related metabolites produced by three *F. proliferatum* strains — harbouring genetic variation within the fumonisin biosynthetic gene cluster — grown on PDA, maize, and rice.

References

- 1) te Brinke, E., Arrizabalaga-Larrañaga, A., Blokland, M. H. (2022). *Analytica Chimica Acta*, 1222, 340039. <https://doi.org/10.1016/j.aca.2022.340039>
- 2) Celma, A. (2023). Screening of pollutants in the environment. *The handbook of environmental chemistry*. Springer. 138. https://doi.org/10.1007/698_2023_1055

2.5 L5 - Mycotoxins in Ethiopian Food Systems: Current Burden and Emerging Biotechnology as a Solution

Abdi Mohammed

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Mycotoxins are toxic secondary metabolites produced by filamentous fungi that contaminate food and feed, posing serious risks to human and animal health and causing substantial economic losses. In Ethiopia, mycotoxin contamination remains a persistent food safety challenge across key staple and cash crops, including maize, peanuts, sorghum, wheat, teff, pepper, and dairy products, with implications for public health, nutrition, food security, and domestic and international trade problems. Available evidence indicates that aflatoxins, fumonisins, ochratoxin A, trichothecenes (including deoxynivalenol), and zearalenone are among the most frequently detected mycotoxins in Ethiopian food crops. However, the emerging mycotoxins such as beauvericin, enniatins, moniliformin, and *Alternaria* mycotoxins have also been reported in different important crops. Among the major mycotoxins, aflatoxins as high as 4500 to 11,900 µg/kg have been reported in peanut and peanut food products. Fumonisin B₁ maximum levels ranged from 6770 to 11,830 µg/kg reported in maize. These contaminants significantly contribute to the dietary exposure of consumers and affecting the public health. There is also evidence that elevated the maximum aflatoxin levels in the export commodities from Ethiopia have led to rejection in international markets.

Globally, different management approaches are employed in mycotoxin mitigation along the production chains. Thus, the conventional management strategies have shown limited effectiveness; recent advances in biotechnology offer promising, sustainable solutions for mycotoxin mitigation along the food value chain. Biological control using atoxigenic *Aspergillus* strains and antagonistic microorganisms has demonstrated substantial reductions in aflatoxin contamination in several African countries, although evidence from Ethiopia remains limited, including the development of fermentation food systems at household levels. In addition, molecular diagnostic tools, including PCR-based identification of toxigenic fungi, marker-assisted breeding for mycotoxin-resistant crop varieties, genomic and metabolomics technologies, and emerging enzymatic and microbial detoxification approaches, provide new opportunities for both pre- and postharvest control. Despite these technological advances, national-scale adoption in Ethiopia is constrained by limited infrastructure, technical capacity, financial resources, and stakeholder awareness. This review synthesizes current evidence on the burden of mycotoxins in Ethiopian food systems and critically examines the potential of emerging biotechnological innovations to drive sustainable mitigation as a solution to enhance farmers' incomes and protect public health within an integrated One Health framework supported by enabling policies and public-private partnerships.

Keywords: Food Safety; Mycotoxins; Biotechnology; Public Health; Food Security; One Health; Ethiopia.

2.6 L6 - Mycotoxins and plant toxins in plant-based drinks – Survey of the German market 2024-2025

Arnold Bahlmann, Christoph Hutzler, Christian Jung, Nicole Lorenz, Benjamin Sachse, Stefan Weigel

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Plant-based milk alternatives have gained relevance in recent years due to the growing popularity of a vegan lifestyle, rising health consciousness and environmental concerns. These drinks can contain mycotoxins and plant toxins, which may pose health risks to consumers. To address this, a highly sensitive LC-MS/MS method was developed to detect these toxins in oat, soy and almond-based drinks. A total of 91 analytes were validated in accordance with EU regulation 2023/2782 and the latest draft of the EURL guidance documents on performance criteria. Among the 67 validated mycotoxins were regulated mycotoxins such as aflatoxins and ergot alkaloids as well as emerging mycotoxins such as *Alternaria* toxins and enniatins. The 24 validated plant toxins included pyrrolizidine and tropane alkaloids, as well as the quinolizidine alkaloid marker lupanine.

Samples of a total of 162 plant-based drinks were taken from the German market in 2024 and 2025. A total of 74 analytes could be detected in at least one drink. Oat drinks contained the highest number of analytes (49), almond the lowest number (28). Risk assessments were carried out for aflatoxin B1 in almond drink, deoxynivalenol and T-2-/HT-2-toxins in oat drink and ochratoxin A in almond drink and soy drink (1). Notably, the tropane alkaloids atropine and scopolamine were repeatedly found in one soy drink product. The highest level found in soy drink was 1,3 µg/kg for the sum of atropine and scopolamine raising concerns of adverse health effects.

The optimized analytical method including sample preparation and LC-MS/MS conditions along with findings from the analysis of samples from the German market and an overview of the risk assessment will be presented.

References

- 1) German Federal Institute for Risk Assessment, 2026. Mykotoxine in Soja-, Mandel- oder Haferdrinks: BfR aktualisiert die Bewertung gesundheitlicher Risiken von Pflanzendrinks anhand neu erhobener Daten; Stellungnahme Nr. 009/2026 des BfR vom 16. Februar 2026. <https://www.bfr.bund.de>

2.7 L7 - Fungi and Mycotoxins Contamination of Selected Aromatic Plants

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Members of the mint order of flowering plants (*Lamiales*) are rich in various polyphenols, and other bioactive compounds that make them valuable medicinal plants as well as an important source of ingredients used in cosmetic, food and beverage industries. However, their quality and safety can be endangered by contamination with toxigenic fungi and their secondary metabolites. The aim of this research was to evaluate mycotoxicological status of selected herbs from the heterogenous mint order of flowering plants acquired from the local store in Croatia. We have analysed plants, leaves or flowers of *Fraxinus excelsior* (Common ash), *Hyssopus officinalis* (Hyssop), *Melissa officinalis* (Lemon balm), *Plantago lanceolata* (Ribwort Plantain), *Rosmarinus officinalis* (Rosemary), *Salvia officinalis* (Common sage), *Satureja montana* (Winter savory), *Thymus vulgaris* (Garden Thyme) and *Verbascum thapsus* (Mullein). Five samples of each herb were used in the study. Enumeration of moulds in herbs was made with standard ISO 21527 method, while moulds were identified with relevant fungal keys. Mycotoxins (aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2/HT-2 toxins and zearalenone) in samples were detected with commercial immunoassays, and confirmed with High Performance Liquid Chromatography Methods.

Results of this study showed presence of the low levels of moulds from the genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* in all analysed herbs. Tested mycotoxins were detected in all samples in diverse range of concentrations. Quantitatively most common mycotoxin was deoxynivalenol, and their presence was detected in the range from 67.39 to 34912.46 µg/kg. Among other mycotoxins, aflatoxins were present in the range from 0.94 to 25.15 µg/kg, fumonisins in the range from 1.61 to 73.34 µg/kg, ochratoxin A in the range from 4.55 to 23.56 µg/kg, T-2/HT-2 toxin in the range from 51.21 to 209.76 µg/kg and zearalenone in the range from 47.16 to 876.23 µg/kg. Our study highlights the importance of quality control testing of herbs and their extracts, evaluation of potential risks and development of appropriate measures to minimize fungal contamination of plants and thus minimize risks of potential toxic effects of their use. This study was funded by the European Union NextGenerationEU and supported by the Ministry of Science and Education of the Republic of Croatia through the project NPOO 15 of Croatian Veterinary Institute entitled "Comprehensive Research on the Impact of Plant Extracts against resistant *Staphylococcus* (CRISP)"

Keywords: plants, moulds, mycotoxins

2.8 L8 - Seasonal mycotoxin exposure and risks in Swiss children based on urinary biomonitoring

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Mycotoxins are frequent food contaminants and may pose health risks, yet seasonal variability in children's internal exposure and related risks remains poorly characterized because most HBM studies rely on a single time point. We characterized seasonal patterns and risk-relevant exposure to mycotoxins among Swiss schoolchildren using repeated urinary biomonitoring.¹

In a longitudinal study, 206 primary schoolchildren in Valais, Switzerland provided 795 spot urine samples across four one-week rounds (January–June 2024). Samples were analysed by LC–MS/MS for a targeted panel of regulated and emerging mycotoxins (including deoxynivalenol; aflatoxins B1, B2, G1, G2, and M1; fumonisins B1 and B2; enniatins; beauvericin; and sterigmatocystin). AFB1, ochratoxin A (OTA), and zearalenone (ZEA) were quantified and used for intake estimation and HBM-based risk characterization. Daily intakes were estimated via urinary back-calculation using literature-based ranges of urinary excretion fractions and age-specific daily urine volume assumptions (sensitivity analyses). Risk was evaluated by comparing estimated ZEA intakes with the tolerable daily intake (TDI) and margins of exposure (MOE) for AFB1 and OTA.

Overall detection frequencies were 63.9% (ZEA), 22.5% (OTA), and 9.2% (AFB1) and varied across assessment periods. Detection frequencies increased toward early summer for OTA (12.3% to 33.2%) and AFB1 (8.7% to 15.6%), while ZEA remained comparatively stable, indicating seasonal differences more evident in detection frequency than in concentration distributions. Intraclass correlation coefficients indicated substantial within-child variability for ZEA (ICC≈0) and AFB1 (ICC=0.30), but more stable exposure ranking for OTA (ICC=0.77). Median estimated daily intake (EDI) was 70.11 ng/kg/day (IQR 54.24–109.80) for AFB1, 60.28 ng/kg/day (IQR 44.52–85.79) for OTA, and 7.36 ng/kg/day (IQR 4.84–11.42) for ZEA. No sample exceeded the TDI for ZEA (0/511; 0%). In contrast, all detected AFB1 (73/73; 100%) and OTA (180/180; 100%) samples had MOE values <10,000, indicating a potential health concern and supporting the need for refined exposure assessment and mitigation. These findings show that single spot urine samples may misclassify exposure to some mycotoxins and that repeated seasonal sampling strengthens exposure and risk characterization in children.

References

- (1) Cramer, B.; Visintin, L.; Maris, E.; Kuhn, M.; Degen, G. H.; Turner, P. C.; Humpf, H.-U.; De Saeger, S. Human Biomonitoring of Mycotoxins: Key Challenges and Future Directions. *Mycotoxin Res* 2026, 42 (1), 13. <https://doi.org/10.1007/s12550-025-00612-2>.

2.9 L9 - Mycotoxin Profiles of *Alternaria* spp. in Oat Grains under Different Water Activity and Temperature Conditions

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Oats are vulnerable to fungal contamination, with *Alternaria* spp. being one of the most prevalent toxigenic fungi (1). These species pose a food safety risk due to their ability to produce mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), and tentoxin (TEN). In response, the European Commission (2) has established indicative levels of 2 µg/kg for AOH, 2 µg/kg for AME, and 500 µg/kg for TeA in infant cereal-based foods. This study evaluates the mycotoxin production potential of *Alternaria alternata* and *Alternaria arborescens* directly on γ -irradiated oat grains under a full factorial design with different temperature (5–40 °C) and water activity (a_w 0.90–0.98) conditions. Four toxigenic strains isolated from oats (three *A. alternata* and one *A. arborescens*) were incubated for 30 days, after which mycotoxin production was quantified by UPLC-MS/MS. Maximal mycotoxin production was detected at high a_w (0.98) and was severely restricted at low a_w (0.90). TeA was detected between 10 and 35 °C, with optimal production at 25 °C (357–800 mg/kg for *A. alternata* and 357 mg/kg for *A. arborescens*). AOH and AME were produced between 15 and 30 °C for both species. For *A. alternata*, maximum AOH was observed at 30 °C (92–398 mg/kg) and maximum AME at 25 °C (17–175 mg/kg), whereas for *A. arborescens*, optimal AOH production was at 20 °C (92 mg/kg) and at 25 °C for AME (1067 mg/kg). TEN was produced between 10 and 30 °C by *A. alternata*, with optimal production at 25 °C (1.25–1.8 mg/kg), while only low TEN production was observed for *A. arborescens* at 20 °C (≤ 0.05 mg/kg). These results are relevant during the oat grain-filling, when kernels maintain high moisture content ($a_w \geq 0.98$) and temperatures ranging between 20 and 30 °C, which align with optimal mycotoxin production in the evaluated *Alternaria* spp. isolates. Notably, the TeA to AOH/AME production ratio in oats was <10-fold, contrasting with the 250-fold ratio used in Commission Recommendation 2022/553 (2), suggesting that even lower levels of AOH and AME may present a greater toxicological risk in oat-based products.

Keywords: *Alternaria alternata*; *Alternaria arborescens*; oat grains; alternariol; alternariol monomethyl ether; tenuazonic acid; tentoxin.

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2.10 L10 - Skin microbiota of processing tomato fruits in relation to *Alternaria* black spot and mycotoxin contamination

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Tomato is one of the most widely cultivated vegetable crops worldwide (1). However, its high susceptibility to *Alternaria* is of particular concern because species of this genus produce mycotoxins that pose potential health risks (2). Processed tomato products have repeatedly been reported as contaminated (3-5), and the European Recommendation 2022/553 encourages identifying the causes of contamination to support future prevention and control measures (6). Yet, little is known about the factors influencing contamination in field-grown processing tomatoes. This study aimed to evaluate the relation between the fruit-associated microbiota of processing tomatoes at harvest and during postharvest handling, and contamination by *Alternaria* and its toxins. At harvest, fruits classified as healthy or diseased were sampled in the field. Several batches of tomatoes were also collected over a 24-hour storage period in skips, to assess the evolution of contamination during this period. *Alternaria* contamination was assessed through visual symptom scoring, qPCR targeting *Alternaria* DNA, and LC-MS/MS quantification of six *Alternaria* toxins (7). The tomato-associated microbiome was characterized by 16S rRNA gene and ITS metabarcoding to profile bacterial and fungal communities, respectively. Tenuazonic acid was the predominant toxin detected, followed by alternariol and alternariol monomethyl ether. Tentoxin, altenuene, and altertoxin I occurred only occasionally but sometimes reached high concentrations. Visual disease severity, *Alternaria* DNA levels, and mycotoxin contamination did not consistently correlate at either the harvest or postharvest stages; notably, some fruits with mild symptoms exhibited high *Alternaria* DNA levels and substantial toxin accumulation. *Alternaria* DNA decreased during the 24-hour storage period, suggesting a beneficial impact of skip-associated microbial communities. Microbiome comparisons between healthy and diseased fruits, and between field and skip samples, identified potential *Alternaria* antagonists. These included for example lactic acid bacteria from the genera *Lactococcus* and *Leuconostoc*, as well as *Pantoea*, *Bacillus*, *Vagococcus*, *Gibellulopsis*, and *Wickerhamomyces*. Several strains of these taxa have been previously reported as antagonists, while others emerge here as new candidates. Isolates from these genera warrant evaluation individually or in synergistic consortia as biocontrol agents to reduce *Alternaria* growth and toxin production. *Bacillus* strains belonging to the *amyloliquefaciens* operational group were isolated from tomato fields, and some laboratory experiments have demonstrated promising activities against *Alternaria*.

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2.11 L11 - Growth Modelling of T-2 and HT-2 Toxin-Producing *Fusarium* spp. in Oat-Based Medium

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Oat crops are vulnerable to colonization by mycotoxigenic fungi such as *Fusarium sporotrichioides* (FS) and *Fusarium langsethiae* (FL), which are the main producers of T-2 and HT-2 mycotoxins. The European Commission Regulation 2023/915 (1) has established a maximum level of 1,250 µg/kg for the sum of T-2 and HT-2 toxins in unprocessed oat grains. Recently, FL has been detected in southern Europe, where it is uncommon, raising concerns about its spread and mycotoxin risk. This study determine the boundary growth conditions and T-2 and HT-2 production of FS y FL strains isolated from Spanish oat grains under different temperature and water activity (a_w) conditions. Five strains of each species were inoculated onto oat-based agar plates and incubated for 28 days at temperatures ranging from 5 to 40 °C and at a_w levels of 0.87, 0.90, 0.94, and 0.98. Fungal growth was monitored by measuring colony radius and was fitted using a simplified Baranyi and Roberts equation (2) to estimate biokinetic parameters, while secondary modeling, using the Cardinal Model with Inflection (3), was applied to determine cardinal growth parameters, including minimum, optimum, and maximum temperatures (T_{min} , T_{opt} , T_{max}), minimum and optimum a_w ($a_{w(min)}$, $a_{w(opt)}$), and the optimal radial growth rate (μ_{Ropt}). Toxin analysis was performed using HPLC-FLD. Secondary growth models predicted that FS may grow between -2 °C and 40 °C, with an optimum around 27 °C (7.8–9 mm/d), while FL may grow between 0 and 35 °C, with an optimum of 24–27 °C (7 mm/d). Both species showed an $a_{w(opt)}$ of 0.98, with minimum $a_{w(min)}$ of 0.89 for FS and 0.90 for FL. These models highlight that FS grows faster and tolerates a wider temperature range, whereas FL requires slightly higher moisture to develop optimal production of T-2 and HT-2 toxins occurred at high a_w (0.98). T-2 production was highest at 15 °C, reaching approximately 12 mg/kg for FS and 5 mg/kg for FL, while HT-2 production peaked at 20 °C, with around 2 mg/kg for FS and 4 mg/kg for FL. The study provides valuable parameters for predictive models to assess the potential occurrence of T-2 and HT-2 toxins in oats under different environmental scenarios, supporting risk management and mitigation strategies in cereal production.

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2.12 L12 - Differential responses of *Fusarium avenaceum* strains to oxidative stress: impacts on growth and mycotoxin yield.

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Oxidative stress, primarily triggered by host-generated reactive oxygen species (ROS), is a pivotal factor shaping plant-pathogen interactions. As such, pathogens must effectively counteract ROS to ensure their survival and pathogenicity. Over the years, several studies have highlighted the role of ROS in the accumulation of major mycotoxins such as type B trichothecenes (1) or aflatoxins (2). However, data on emerging mycotoxins remain scarce.

Fusarium avenaceum, a notorious fungal pathogen of cereal crops, is one of the main producers of enniatins, a class of emerging mycotoxins posing threat to human and animal health (3). In the recent years, several studies have pointed out the considerable intraspecific diversity in *Fusarium avenaceum* both genetically and phenotypically (4), influencing its adaptability and pathogenic potential. In this study, we investigate the response of multiple *F. avenaceum* isolates to hydrogen peroxide (H₂O₂), a key ROS molecule involved in host defence signalling. 12 *F. avenaceum* strains were grown in both liquid and solid media supplemented or not with H₂O₂, and were assessed for growth and enniatins production. *F. avenaceum* susceptibility to H₂O₂ was found to be highly strain-specific. Exposure to H₂O₂ led to a reduction in growth consistent with a delay in spores' germination, and enniatin production was either enhanced or reduced depending on the isolate. These data were also supported by a transcriptomics analysis targeting enniatins biosynthetic genes. Moreover, the expression of oxidative stress-related genes was also found to be activated by H₂O₂, consistent with the high sensitivity of *Fusarium avenaceum* isolates to oxidative stress compared to other *Fusarium* species causing Fusarium Head Blight. Comparison of enzymatic equipment related to oxidative stress response is ongoing, and could help better understanding these differences in H₂O₂-response between *F. avenaceum* strains. These findings highlight the complex interplay between genotype and stress response in *F. avenaceum*. Understanding the oxidative stress response of *F. avenaceum* contributes to deciphering its pathogenic mechanisms, and could potentially lead to targeting specific vulnerabilities in integrated disease management strategies.

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2.13 L13 - Mycotoxin threats in a changing climate: effects of interacting abiotic factors on fungal growth and OTA/CIT production in rice

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Rice is a staple food for more than half of the global population and is highly sensitive to environmental fluctuations (1). Climate change scenarios predict increased temperatures, elevated atmospheric CO₂ concentrations, and altered moisture regimes, particularly in Mediterranean regions identified as climate “hot spots” (2, 3). Such changes may alter the ecology of spoilage fungi and shift patterns of mycotoxin contamination along the rice supply chain.

This study investigated the effects of interacting climate-related abiotic factors on fungal growth and mycotoxin production in rice systems. Two mycotoxigenic species were evaluated: *Penicillium nordicum* (producer of ochratoxin A, OTA) and *Penicillium citrinum* (producer of citrinin, CIT). They were tested under combinations of temperature (25 and 27 °C), water activity (a_w 0.93 and 0.96), and CO₂ concentration (400 and 1000 ppm). Growth was assessed *in vitro* on rice-based and yeast extract sucrose (YES) media, while mycotoxin accumulation was determined in inoculated media and raw rice grains after 14 days of incubation under controlled atmospheric conditions.

The experimental design allows assessment of interactive effects of a_w x temperature x CO₂ on fungal behavior and mycotoxin production. Preliminary results indicate that water availability is a key driver of growth dynamics, while elevated CO₂ interacts with temperature to modulate OTA and CIT production in rice grains. These findings highlight the potential for climate-driven shifts in mycotoxin contamination risk in post-harvest rice systems.

Understanding how interacting abiotic stressors influence fungal ecology is essential for developing predictive models and strengthening surveillance strategies to safeguard rice-based food chains under future climate scenarios.

Keywords: climate change, storage fungi, ochratoxin A, citrinin, *Penicillium*

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2.14 L14 - New PKS and metabolites putatively involved in ochratoxin A accumulation by *Penicillium nordicum* in NaCl-rich environments

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Penicillium nordicum is the main ochratoxin A (OTA)-producing fungus in dry-cured meat products. The OTA accumulation by *P. nordicum* is enhanced at high salt concentrations, since it is considered as an adaptive strategy to that environment. However, the genetic and metabolic mechanisms underlying this adaptation remain poorly understood. The genes involved in OTA biosynthesis include a polyketide synthase (PKS), although other PKS outside the cluster might also influence OTA production in *P. nordicum*.

This study aimed to evaluate, at the molecular level through gene expression and metabolomics, potential new PKS involved in OTA biosynthesis and the metabolites related to this mycotoxin at different NaCl concentrations.

Optimized protocols were designed for gene expression analysis of five genes that encoded related PKS that were previously reported to be overexpressed in dry-cured meat products (*n2*, *n3*, *n4*, *n5* and *n6*; [1]). Their expression was tested in two strains of *P. nordicum* (Pn15 and Pn856) grown in CYA medium with different salt concentrations (control without salt, 70, and 150 g/L). After incubation, fungal mycelia were used for gene expression analyses and metabolomics.

The results indicated that *n3*, *n4* and *n6* genes seem to be related to OTA biosynthesis in Pn856 whereas *n2* suggests a role in OTA degradation. The lack of correlations between the genes studied and OTA in Pn15 could be due to the low OTA levels detected and its quick transformation into other compounds. The metabolites coniferyl alcohol and 6-hydroxy-8-methoxy-3-methylisochroman were correlated to OTA in both strains. The 3-methylbutanoic acid and cephalosporolide E might be used as indicators to predict OTA. Overall, these findings show a deeper understanding of the regulatory complexity of OTA in *P. nordicum*. Our study provides potential genetic and metabolic biomarkers to develop targeted strategies for mitigating mycotoxin contamination in NaCl-rich foods.

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2.15 L15 - Quantifying interaction dynamics among mycotoxigenic fungi

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In recent years, the contamination of crops by multiple mycotoxins has become an increasing concern, often associated with climate change (1). Particularly cereals are highly susceptible to colonization by toxigenic fungi including *Aspergillus flavus*, *Fusarium verticillioides*, and *F. graminearum*, each producing distinct mycotoxins that threaten food and feed safety (2). While individual responses of these species to environmental factors are well studied, less is known about their interactions when co-occurring, especially under varying climatic conditions (3). Field evidence also indicates that fungal co-occurrence during the growing season can shape mycotoxin contamination patterns in maize (4).

To address this gap, a small-scale *in vitro* workflow was developed to quantify growth kinetics and competitive outcomes among these three mycotoxigenic fungi across a temperature gradient from 10 to 45 °C. The workflow combines a microplate co-culture assay with spectrophotometric monitoring and growth-curve modelling. To distinguish species specific contributions, a qPCR-based approach was used, enabling quantitative estimation of individual species abundance within the total biomass. The results indicated that co-culture growth optima shifted towards warmer temperatures compared with monocultures, and interaction effects changed across temperatures and species combinations. Co-culture composition was temperature sensitive, with *A. flavus* contributing an increasing fraction of total biomass at higher temperatures and both *Fusarium* species contributing relatively more at lower temperatures.

These findings provide new quantitative insights into fungal co-occurrence dynamics under changing environmental conditions. The proposed small-scale workflow represents an innovative and flexible approach for investigating the ecology and competitive behaviour of mycotoxigenic fungi under controlled environmental scenarios. The resulting interaction data can support improvements in predictive mycotoxin risk models, which have become essential for decision-making and contamination risk management (5).

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2.16 L16 - Genetic characterization and geographical variation in mycotoxin profiles of *Fusarium culmorum* from wheat in South of Italy

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Fusarium culmorum is a major pathogen of cereal crops, worldwide. Recently, this species has been proved to be able to produce NX toxins, the most recently discovered group of trichothecenes. These new type A trichothecenes, specifically NX-2, were originally reported only in Northern American by strains belonging to the *Fusarium graminearum* species complex. In the last years, NXs were also detected in vitro cultures of *F. culmorum* strains isolated from wheat across Europe and Asia.

In a two year survey, aimed to investigate the incidence of the Fusarium crown rot (FCR) in Southern Italian wheat crops, we have isolated and detected a wide population of *F. culmorum*. A total of 110 selected strains, isolated across 6 regions of South of Italy, were analyzed in vitro for their capacity to produce 8 mycotoxins [NX2 M1, NX2, NX3, deoxynivalenol (DON), Nivalenol (NIV), 3 AcDON, 15 AcDON, and zearalenone (ZEA)]. Deoxynivalenol (DON) and acetyl DON derivatives (3 AcDON, and 15 AcDON) were the toxins most commonly produced, varying in frequency across regions. Several toxins (NX2, NX3, NX2 M1, NIV, ZEA) exhibited clear regional associations, with Campania and Basilicata showing the largest number of unique toxin profiles, and Sicilia and Calabria the lowest.

Representative strains of this population has been analyzed genomically for detecting biosynthetic gene clusters related to mycotoxin production and built genetic relationship among the *F. culmorum* strains. Finally, a subset of around 70 strains was analysed for the presence of two TRI1 Single Nucleotide Polymorphisms (SNPs) previously described in *F. graminearum* as determinants of the biosynthesis of NX type trichothecene. In *F. graminearum*, these SNPs discriminate the NX non producing strains from the producing ones. However, statistical correlation analysis performed on our *F. culmorum* population revealed no association between none of the two SNPs and the production of any mycotoxin measured, including the NX related metabolites NX2 M1, NX2 and NX3. These results demonstrate that, unlike in *F. graminearum*, the TRI1 SNPs tested do not predict NX production in the *F. culmorum* strains studied. This finding is fully consistent with studies from other countries, which likewise report that TRI1 variation shows no functional link to NX biosynthesis in this species. Consequently, the biosynthetic determinants of NX production in *F. culmorum* remain unresolved, and the chemotype diversity observed among isolates appears to be shaped by factors other than the TRI1 polymorphisms examined here. Together with the regional toxin patterns detected in this study, these results highlight distinct geographic structuring of secondary metabolism within Italian *F. culmorum*, suggesting potential ecological, climatic, or agronomic influences on chemotype distribution.

2.17 L17 - Detection of Secondary Metabolite Biosynthetic Genes Across the *Fusarium oxysporum* Species Complex

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The *Fusarium oxysporum* species complex (FOSC) comprises some of the most globally pervasive soil-borne fungal pathogens, recognized for their impact on crop yield, quality, and food safety due to the production of toxic secondary metabolites. Within FOSC, strains are traditionally classified into *formae speciales* based on host specificity; however, many remain poorly characterized, limiting our understanding of their evolutionary relationships and pathogenic potential.

In this study, we investigated more than 150 *F. oxysporum* strains isolated from diverse plant hosts and geographical regions to evaluate their intraspecific molecular biodiversity. A genome-wide SNP analysis was conducted on all isolates to resolve population structure and identify genetic signatures associated with host specificity and geographic origin. In parallel, we used Big-Map to profile across all genomes the abundance of biosynthetic gene clusters (BGCs) involved in secondary metabolites production. This approach enabled the detection, annotation, and cross-strain comparison of core and accessory BGCs potentially linked to virulence and ecological adaptation.

Among the detected BGCs, we focused on those related to fumonisin and beauvericin due to their relevance in toxigenic *Fusarium* species and their implications for food safety. By examining their distribution, sequence variability, gene composition (presence or absence of key biosynthetic genes), and overall synteny, we compared the structural diversity of these clusters across the different *formae speciales*. Notably, some isolates within FOSC were found to harbor the complete fumonisin biosynthetic gene cluster, whereas others possessed only partial sub-sets of the cluster, indicating a heterogeneous distribution and possible independent evolutionary trajectories of fumonisin-related pathogenic traits.

Overall, the genomic analyses highlight major patterns of diversity within FOSC and improve the resolution of relationships among *formae speciales*. Future integration of metabolomic data will further support marker identification and the development of strategies for crop protection and food-safety management.

2.18 L18 - Shuffling of the patulin biosynthetic gene cluster in *Penicillium roqueforti*

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Fungi are known to produce many chemically diversified and biologically active metabolites [1, 2]. However, the ecological roles of these compounds within specific niches remain incompletely understood. *Penicillium roqueforti* is a very interesting case study as this species thrives in diverse anthropized niches, ranging from lumber to silage, but also in food systems, where it acts as a technological auxiliary in blue cheese production or as a contaminant. Across these environments, it can produce a wide range of secondary metabolites, including mycotoxins. Although strong variations in the production of some mycotoxins have been documented among strains belonging to distinct populations [3], the case of patulin remains unresolved. We therefore screened 200 fully assembled and annotated *P. roqueforti* genomes for the biosynthesis gene cluster involved in patulin production and identified 12 different gene organizations. Surprisingly, this included a complete pathway in four *P. roqueforti* genomes. The other eleven clusters contained different gene rearrangements or a truncated version of the cluster. A similar truncated pathway was also identified in another species of the *Roquefortorum* section, but three others had the complete pathway, namely *P. psycrosexualis*, *P. paneum* and *P. carneum*. Comparative analyses revealed that strains possessing a complete patulin cluster exhibited a gene organization identical to that of the well-characterized *P. expansum* cluster. Nevertheless, none of the *P. roqueforti* strains with the complete patulin cluster produced patulin after growth on classical rich medium or on typical substrates mimicking the different environmental niches, such as apple sauce agar. On the contrary, patulin was consistently produced by *P. expansum* in all tested conditions. We therefore evaluated gene expression levels in *P. roqueforti* by targeting two key genes in the patulin biosynthetic pathway to confirm that the patulin cluster is non-functional in this species.

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2.19 L19 - Population genomics and aflatoxin production of *Aspergillus flavus* and *Aspergillus oryzae* from China

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Aspergillus flavus is usually considered a dangerous fungus that produces aflatoxins and infects humans; while *A. oryzae* has been used in food fermentation for thousands of years and is generally recognized as safe. However, the two species are indistinguishable phenotypically and molecularly (1), triggering the debate on their separation as distinct species. In previous population genomic studies of *A. flavus/oryzae*, Chinese strains were poorly represented (2), though *A. oryzae* is more widely used and has a longer domestication history in China (3). Here we performed phylogenomic, comparative genomic, and aflatoxigenic studies on 221 *A. flavus/oryzae* strains from diverse geographical and ecological sources in China, together with reference strains representing the maximum genetic and ecological diversities of the species documented so far in the world. Four distinct lineages (Lineages 1-4) were recognized from the strains employed and almost all the non-aflatoxigenic fermentation strains conventionally identified as *A. oryzae* were clustered in a monophyletic lineage (Lineage 4). Surprisingly, the ex-type strains of *A. flavus* and *A. oryzae* clustered together in Lineage 4. The strains with non-fermentation origins located in Lineage 4 were also non-aflatoxigenic. Though 66.4% of the Lineage 4 strains harbor an intact aflatoxin biosynthesis gene cluster, most of them share the same loss-of-function mutations in genes *hypA*, *hypB*, *aflJ*, and *aflT* of the cluster. The results support the idea that *A. flavus* and *A. oryzae* are biologically conspecific and the latter represents a domesticated ecotype of the former. We propose to treat *A. oryzae* represented by Lineage 4 as a special form (*forma specialis*, f. sp.) of *A. flavus* and name it *Aspergillus flavus* f. sp. *oryzae*. We provide 15 single copy orthologue genes that can be used to identify the *A. oryzae* form at an accuracy of >99% each.

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2.20 L20 - Pathogenicity-Associated Metabolic Differentiation in *Trichoderma afroharzianum*

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Trichoderma afroharzianum is widely recognized as a beneficial fungus used in biological control and plant growth promotion (1). However, recent observations have identified certain strains as emerging pathogens causing ear and stalk rot in maize, challenging the traditional perception of this species (2). The mechanisms underlying this functional shift remain poorly understood, particularly with respect to the role of secondary metabolism.

In this study, LC–HRMS analysis was employed to investigate the association between pathogenicity and metabolite production in *T. afroharzianum*. A diverse collection of 110 *Trichoderma* isolates, representing pathogenic, moderately pathogenic, and non-pathogenic phenotypes, was evaluated through both *in vitro* maize culture and *in planta* experiments. A targeted panel of 18 metabolites covering multiple chemical classes, including polyketides, terpenes, volatile organic compounds, and plant hormone-related compounds, was assessed.

Results revealed clear metabolic differentiation linked to pathogenicity. While a core set of metabolites was consistently detected across isolates, pathogenic strains exhibited significantly higher abundance of specific metabolites and distinct compositional shifts, particularly within symptomatic maize tissues. Key metabolites such as chrysophanol, harzianopyridone, trichodiene, and abscisic acid were strongly associated with disease symptoms and increased virulence. In contrast, classical mycotoxins including trichodermin, gliotoxin, and viridin were not detected, indicating that pathogenicity is not driven by known toxin classes.

Comparative analyses between *in vitro* and *in planta* conditions further demonstrated that metabolite production is strongly influenced by host interaction and tissue type, with the highest metabolite levels observed in symptomatic cob tissues infected by highly pathogenic strains. These findings support the concept that pathogenicity in *T. afroharzianum* is governed by a combinatorial and context-dependent metabolic strategy rather than the presence of single virulence factors.

Overall, this study provides a metabolomic framework for distinguishing pathogenic from beneficial *Trichoderma* strains and highlights the importance of strain-level risk assessment in the application of microbial biocontrol agents.

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2.21 L21 - Going with the wind...and other weather variables to predict mycotoxin risk

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Mycotoxins are toxic secondary metabolites produced by certain fungi, particularly from the genera *Aspergillus*, *Fusarium*, and *Penicillium*. These compounds can contaminate a wide range of agricultural products, including grains, nuts, seeds, and fruits, during various stages of production, from cultivation to storage. Mycotoxins pose significant risks to human and animal health, as they can cause acute poisoning, long-term health effects, and economic losses in agriculture (García-Díaz et al., 2020). It is estimated that a substantial portion of crops, ranging from 25% to 80%, may be contaminated with mycotoxins globally, posing a persistent challenge for agricultural professionals and underscoring the critical need for effective management strategies. (Kovač et al., 2018). Predicting mycotoxin concentrations before harvest would greatly benefit feed and food production (Inglis et al., 2024). Since mycotoxin levels correlate with weather (Platzer et al., 2025) - and weather can be forecast - it is possible to build predictive models. By combining weather and phenological data we developed models that forecast the probability of presence of mycotoxins above or below given thresholds in different types of samples. Meteorologic conditions directly influence the growth of mycotoxigenic fungi and their subsequent toxin production. By further integrating mycotoxin phenological data, we achieved statistically significant and accurate predictions while addressing the increased complexity of the dataset through a machine learning algorithm. Our ML models use as input daily weather data, classified samples into "High" or "Low" risk categories based on a given threshold related to each mycotoxin. We used ERA5 weather data (Hersbach et al., 2020) - providing information for temperature, relative humidity, dewpoint, precipitation rate and wind speed - alongside the dsm-firmenich mycotoxin survey (Gruber-Dorninger et al., 2019) which provide the risk category and geographic locations of each sample. We set strict criteria to refine our datasets based on each sample specific aspects, harvest time for each country, storage time of each sample. Close 1/3 of the initial samples were excluded (the number differ per mycotoxin dataset). We assess our models through classification evaluation metrics and statistical tests, excluding models that were not passing both accuracy and statistical significance threshold. Our final models will aid in planning preventive measures to mitigate mycotoxin contamination before it occurs.

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2.22 L22 - An Integrated AI-Based Text-Mining Pipeline for Automated Extraction of Mycotoxin Occurrence and Analytical Data from Scientific Literature

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The literature on mycotoxins is growing exponentially due to the increasing number of identified compounds¹ and the wide variety of food and feed matrices that can be contaminated. However, given the volume and complexity of this literature, no integrated pipeline currently enables the fully automated and structured extraction of analytical data specific to mycotoxins from full-text scientific articles. To address this issue, we developed an artificial intelligence (AI)-based text-mining pipeline in Python to enable the automated extraction of key information from scientific articles. The novelty of this work lies in the integration of semi-automated literature retrieval, hybrid rule-based and AI-driven full-text analysis. Thus, it prioritizes rule-based methods for pattern defined tasks and uses AI for linguistically complex extraction, limiting the resources need, abbreviation standardization, and structured data extraction into a single reproducible pipeline dedicated to mycotoxin analytical and occurrence data.

We first applied this pipeline to three *Fusarium* toxins: enniatins, beauvericin, and moniliformin, in food and feed matrices. The workflow of the pipeline is organized into several clear and independent stages. First, articles are identified using defined keyword combinations, resulting in the retrieval of 2,420 unique articles from the Scopus, PubMed, PubTator, and Web of Science databases. The publications were then screened manually and AI driven based on their titles and abstracts according to predefined inclusion criteria, and 215 relevant articles were retained for further analysis. Full-text analysis was conducted using a hybrid approach combining rule-based methods and artificial intelligence. Several AI models were evaluated to determine the most effective one for feature extraction. Model performance was assessed using a dedicated training set and an independent test set to evaluate robustness and enable objective comparison between models based on standard performance metrics. The extracted features included key data such as targeted mycotoxins, matrices, extraction procedures, analytical and quantitative methods, and performance parameters including limits of detection (LOD), limits of quantification (LOQ), and reported occurrence levels. The standardized information was then processed using a locally deployed large language model (LLM) to extract and structure all targeted data into organized data tables.

This pipeline will subsequently be extended to cover all regulated and emerging mycotoxins identified in MycoCentral database (1), and the extracted structured data will be integrated into the MycoCentral database to support the integration of harmonized data and provide open data access, for research and risk assessment purposes.

Keywords: AI, Mycotoxins, Text mining, Occurrence, Database

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2.23 L23 - A novel approach for predicting aflatoxin B₁ production using regression models and whole-cell biosensors in moldy maize and peanut kernels

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Aspergillus flavus infection and subsequent aflatoxin B₁ (AFB₁) contamination represent major causes of post-harvest losses in maize and peanuts, underscoring the urgent need for sensitive and scalable early detection strategies. Thus, this study aimed to develop a transcriptome-guided whole-cell biosensor array coupled with machine learning regression models for quantitative prediction of fungal infection stages and AFB₁ levels. (1) A novel whole-cell biosensor array capable of generating time-resolved bioluminescence signals in response to *A. flavus* infection was constructed. This was achieved by identifying eight infection-induced promoters from *E. coli* transcriptomic responses to volatile organic compounds and integrating them into calcium alginate-immobilized bioreporters. (2) Quantitative prediction of infection stages and AFB₁ levels was achieved with high accuracy and robustness across different food substrates and fungal strains. XGBoost consistently outperformed other ensemble machine learning regression models (CatBoost and Random Forest) trained on the time-resolved bioluminescence signals, attaining in internal validation for maize $R^2 = 0.94$ for infection stage prediction and $R^2 = 0.98$ for AFB₁ quantification. External validation using independent *A. flavus* isolates confirmed robust generalization ($R^2 = 0.92$ and 0.91 , respectively). Comparable results were obtained in peanuts (internal validation: $R^2 = 0.94$ and 0.97 ; external validation: $R^2 = 0.92$ and 0.86), demonstrating robustness across different food substrates and fungal strains. (3) The novel transcriptome-guided biosensor demonstrated significantly enhanced predictive accuracy compared to previous biosensors, with feature importance analysis revealing key biological drivers of prediction. This was demonstrated by benchmarking against our previous biosensors constructed from 14 general stress-responsive promoters, which showed that the transcriptome-guided approach yielded superior performance, particularly under external validation conditions. Feature importance analysis further revealed that early host responses including transcriptional regulation and biofilm formation served as key predictive features, providing mechanistic interpretability not attainable with conventional optical or chemical assays. This study establishes a biologically informed, non-invasive, and cost-effective biosensing platform that integrates promoter-level transcriptomic insights with ensemble machine learning. The approach offers a versatile solution for real-time aflatoxin risk assessment and scalable food safety monitoring across diverse agroecosystems, with demonstrated accuracy and robustness in both maize and peanuts.

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2.24 L24 - Untargeted HRMS screening of maize: a QC-Based Workflow with Linear and Non-Linear Data Characterisation

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High-resolution mass spectrometry (HRMS) coupled with liquid chromatography has established itself as a powerful platform for the characterisation of small molecules in complex matrices. Through full-scan acquisition, HRMS enables retrospective, untargeted identification of diverse compounds, supporting the identification of relevant analytes during subsequent targeted quantification. However, longitudinal data acquisition is affected by systematic and random variability in signal sensitivity, retention times, and mass accuracy between samples, both within and between analytical batches¹. This variability leads to significant information loss and complicates downstream data processing, posing a central data integration challenge to untargeted workflows.

In this study, 121 maize samples were distributed in 5 batches and analysed using an untargeted LC-HRMS approach. The application of R Bioconductor Package for feature extraction, blank subtraction, peak cleaning as well as alignment between and within analytical batches will be showcased. By integrating structured quality controls (QC), process blanks and long-term reference samples, a practice already established in untargeted metabolomics², we demonstrate how system performance can be monitored and variability characterised over time, enabling merging of detected features in one results table for subsequent processing. Non-linear dimensionality reduction approaches (t-SNE and UMAP) were compared with principal component analysis (PCA) for exploratory data characterisation.

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2.25 L25 - NAMs for Food Safety: *In Silico* Modelling of Bacterial Membranes to Explore Mycotoxin Bioavailability in the Intestinal Compartment

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Mycotoxins are naturally occurring xenobiotics that enter the body through contaminated food and become systemically available through the absorption in the gastrointestinal tract. Their bioavailability clearly depends on their physicochemical properties, but also on the host metabolism and on the complex interactions in the intestinal compartment. Recent studies support that physiologically occurring gut bacteria can modulate mycotoxin bioavailability (1) even though much remains to be clarified pertaining the role of the microbiome in regulating mycotoxins-host interactions (2). Hypothesizing that part of the compounds could be physically “trapped” in the microbiome mass, this would have great implications for the development of *in vitro* models predictive of toxicity stemming from foodborne exposures. Considering the wide structural diversity of molecules potentially involved, we started developing an *in silico*-based screening approach and established a model to investigate mycotoxins – microbial membrane interactions at the molecular level. As membrane models are scarce in comparison to protein targets, the initial approach was built on the outer bacterial membrane of *E. Coli*. Progressively increasing the degrees of complexity of the bacterial lipopolysaccharide (LPS) membrane systems, we developed models reconstructing the lipid A layer of the bacterial membrane and included the oligosaccharide chains on the surface in a subsequent step.

Molecular dynamic simulations were carried out to obtain the penetration, permeability behavior and residence time of the selected mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), fusaric acid (FA), deoxynivalenol (DON), altenuene (ALT) and tenuazonic acid (TeA). By comparing the two simulation layouts we could highlight the importance of the oligosaccharide layer, whose presence drastically reduces the permeability of the mycotoxins and returns interaction behaviors clearly retracing experimental data available in literature (3). Notably, DON, FA and AME were still able to penetrate through the hydrophilic oligosaccharide barrier, suggesting a higher bacterial membrane interaction and potential implications for the design of *in vitro* experiments reproducing the complex pathophysiology of the intestinal compartment.

Overall, this study supports that bacterial membranes could modulate mycotoxin residence in the gut and consequently influence their bioavailability. The computational modelling framework is not limited only to the *E. Coli* membrane system, but can be applied to similar biological barriers, enabling the study of foodborne xenobiotics interactions across various physiologically relevant membranes.

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2.26 L26 - Novel insights into the toxicological profile of *Alternaria infectoria* mycotoxins

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Fungal contamination of food is a global concern. Species of the genus *Alternaria* produce a wide range of structurally diverse mycotoxins, some of which may pose a significant health risk for consumers. Due to insufficient toxicological and occurrence data, *Alternaria* mycotoxins remain classified as “emerging mycotoxins”, and no regulatory maximum levels have been determined. To date, research has largely concentrated on the toxicity of metabolites typically produced by *Alternaria alternata*, including tenuazonic acid (TeA), the dibenzo- α -pyrones alternariol (AOH) and its monomethyl ether (AME), and the perylenequinones (PQs) altertoxins I & II (ATX-I & II) and alterperyleneol (ALTP). In contrast, the toxicological relevance of the secondary metabolite profile associated with *Alternaria infectoria*—reported to differ substantially from that of *A. alternata*—has received far less attention. Except for the production of certain PQs (i.e. ATX-I and ALTP), this species typically does not synthesize the well-known *Alternaria* mycotoxins. Instead, these molds produce a variety of infectopyrones, phomapyrones, and novae-zeladins, whose potential impact on consumers health remains difficult to estimate due to the scarcity of available toxicological data [1].

The present study aimed to address this knowledge gap by investigating the chemical composition and toxicological properties of two complex extracts obtained from *Alternaria infectoria* strains (WSD 3.1.1 and WSD 40.2.6). The mycotoxin profiles of the extracts were determined via LC-MS/MS. To assess their potential endocrine-disrupting properties, the alkaline phosphatase assay was performed in Ishikawa cells, while NF- κ B reporter gene assay in THP-1 Lucia monocytes was implemented to investigate the immunomodulatory effects. Finally, genotoxicity was examined in HT-29 cells using the alkaline single-cell gel electrophoresis (COMET assay), performed both with and without formamidopyrimidine DNA glycosylase (FPG) to assess not only DNA strand break induction but also the presence of FPG-sensitive sites, indicative of oxidative DNA damage.

Results of the LC-MS/MS analysis demonstrated that multiple PQs (i.e. ATX-I, ALTP, and a putative ATX-II isomer) were present in the extracts, whereas none of the other mycotoxins typically associated with *Alternaria alternata* were detected. Results from the AIP assay, which was conducted to investigate potential anti-estrogenic effects of the extracts, showed a dose-dependent reduction in AIP activity starting at concentrations of 0.5 μ g/mL for WSD 3.1.1 and 25 μ g/mL for WSD 40.2.6. Both extracts also exhibited strong immunosuppressive properties, leading to a complete suppression of the NF- κ B pathway at 10 and 50 μ g/mL respectively. Finally, COMET assay data indicated DNA damage starting at concentrations as low as 1 μ g/mL for WSD 3.1.1 and 5 μ g/mL for WSD 40.2.6.

In conclusion, the results from this study clearly indicate that, despite the absence of most well-known *Alternaria* mycotoxins, *Alternaria infectoria* extracts are able to exert

several potentially harmful effects *in vitro*. These findings provide a crucial foundation for the comprehensive toxicological evaluation of *Alternaria infectoria* mycotoxins, contributing to a deeper understanding of their potential health risks and guiding future research in this field.

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2.27 L27 - Evaluating the Genotoxic Potential of Emerging Mycotoxins Using Stepwise Approach

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Mycotoxins (MTX) are increasingly detected in food and feed matrices, yet many are neither regulated by the European Commission nor evaluated by the European Food Safety Authority and thus remain under-evaluated. Toxicological characterization is required to support risk assessment on these metabolites. In this context, genotoxicity is of particular relevance as it is associated with important health effects such as cancer.

EFSA's regulatory genotoxicity testing strategy for food and feed assessment (1) includes the Ames test as a first step. This assay is capable of detecting point mutations. However, it requires a substantial workload, time, and quantity of test item (2). This is particularly limiting when considering to evaluate a large set of compounds, especially emerging MTX which are often available only in limited amounts. Miniaturized versions of this assay and bacterial-based screening tests are some alternatives to overcome this disadvantage since they reduce the needed resources while maintaining good concordance with the standard method.

The aim of this work was to evaluate the mutagenic potential of 20 under-evaluated emerging MTX using two bacterial assays. Initially, the SOS/umu test was applied as a screening tool (3). Considering the limited sensitivity of this assay, negative results were validated with a miniaturized version of the Ames test (miniAmes), unless their bibliography in the Ames test was sufficient and reliable. This testing strategy helps identify potentially genotoxic emerging mycotoxins and contributes to filling data gaps in their toxicological profiles.

The obtained results following this testing strategy suggest that 10 MTX have a mutagenic potential: 3-nitropropionic acid, aflatoxicol, asperphenamate, averantin, averufin, butenolide, cyclopiazonic acid, kojic acid, mycophenolic acid and o-methylsterigmatocystin. As for the other MTX (andrastin A, apicidin asperglaucide, aurofusarin, bikaverin, cyclo-(L-Pro-L-Tyr), cyclo-(L-Pro-L-Val), fusaric acid, skyrin and tryptophol) no genotoxic effects were observed.

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2.28 L28 - Early oxidative and mitochondrial stress responses to tenuazonic acid in human esophageal cells

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Tenuazonic acid (TeA) is a frequently occurring *Alternaria* mycotoxin contaminating various food commodities, including cereals, oilseeds, tomatoes and fruits (EFSA 2016). Epidemiological studies reported an association between high dietary exposure to TeA and an increased incidence of esophageal cancer (Dong et al. 1987; Yekeler et al. 2001), underlining the relevance of investigating its effects in esophageal cell models.

In the present study, the dose-dependent effects of TeA were investigated in the human esophageal carcinoma cell line KYSE510. The formation of reactive oxygen species (ROS) was assessed using the dichlorofluorescein diacetate (DCF-DA) assay. Subsequently, mitochondrial integrity and function were examined. Mitochondrial morphology was evaluated by quantification of the MitoTracker signal area after TeA exposure, and mitochondrial respiration was analyzed by determination of the oxygen consumption rate (OCR) using the Seahorse XF assay. Since oxidative stress may activate stress signaling pathways and affect cell cycle progression (Vermeulen et al., 2003), cell cycle distribution was further analyzed by flow cytometry following TeA treatment.

Taken together, the obtained data indicate a sequence of TeA-induced cellular effects in KYSE510 cells at non-cytotoxic concentrations (1 - 10 μ M). An increase in ROS formation was observed after TeA exposure. This was accompanied by impaired mitochondrial respiration and alterations in mitochondrial morphology. Furthermore, a shift in cell cycle distribution towards G₂/M phase arrest was observed. These findings provide mechanistic insights into the cellular effects of TeA in esophageal cells, demonstrating ROS-associated mitochondrial dysfunction and cell cycle modulation at non-cytotoxic concentrations. The results highlight the need for further investigations into the potential role of TeA in esophageal pathophysiology under chronic exposure conditions.

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2.29 L29 - In vitro digestion of lipophilic mycotoxins enniatins and beauvercin

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Enniatins (ENNs) such as ENN A, A1, B and B1 as well as beauvericin (BEA) are lipophilic *Fusarium* mycotoxins frequently contaminating grains and grain-based products. Despite their common occurrence and reported cytotoxicity in cell culture studies, data regarding their fate during human gastrointestinal digestion remain limited and often contradictory. The present study used the standardized protocol for *in vitro* digestion in the upper gastrointestinal tract (GIT) as agreed by the COST INFOGEST network (1) to analyze the bioaccessibility and potential transformation of ENNs and BEA in both naturally contaminated and spiked grain-based matrices. Special emphasis was placed on the behavior of these lipophilic compounds within the aqueous digestion system to account for potential adsorption effects. The bioaccessibility values ranged from $62.8 \pm 7.8\%$ to $72.4 \pm 9.4\%$ across all analytes after *in vitro* digestion in spiked wheat blank flour. However, no transformation of ENNs and BEA could be observed during *in vitro* digestion in spiked blank wheat flour. Similarly, ENN B remained stable during *in vitro* digestion of naturally contaminated rye flour and wheat gluten. A previous study identified masses of ENN B metabolites after *in vitro* digestion (2), and these have also been detected using LC-HRMS despite the absence of quantifiable native ENN B degradation. Since the metabolites were detected in solvent blanks with enzymes as well, this study concludes that ENNs and BEA are stable during upper GIT digestion.

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2.30 L30 - Enniatins and Their Mixture Induce Cytotoxicity in Rainbow Trout (SOB-15) Hepatocytes Through Early Disruption of Heme Biosynthesis

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The increasing incorporation of plant-based ingredients in aquafeeds has elevated the risk of exposure to emerging mycotoxins, including enniatins (ENNs), cyclic hexadepsipeptides produced by *Fusarium* spp [1]. Although ENNB and beauvericin have been associated with anaemia [2], the cellular mechanisms underlying ENN toxicity—particularly regarding heme biosynthesis—remain poorly understood. Moreover, ENNs frequently co-occur in feed, raising concerns about combined effects.

This study evaluated the cytotoxic and mechanistic effects of four major ENN analogues (ENNA, ENNA₁, ENNB, and ENNB₁) using the rainbow trout hepatocyte-derived cell line SOB-15. The enniatins were investigated both individually and as a mixture allowing assessment of potential interactions. Real-time cell analysis (RTCA), based on impedance measurement, was used to assess cell viability/cytotoxicity (0.5–2.5 μM), while protoporphyrin IX (PPIX) accumulation following δ-aminolevulinic acid (ALA) supplementation was measured using autofluorescence to evaluate interference with heme biosynthesis (0.5–1.5 μM). Transcriptomic analysis (RNA-seq) was performed after exposure to the mixture (1.5 μM) at 18 and 30 h.

All analogues induced concentration- and time-dependent cytotoxicity. At 1.5 μM, ENNA and ENNB₁ produced earlier and stronger effects than ENNB, while the artificial mixture showed enhanced toxicity compared to most individual compounds. Importantly, reductions in PPIX levels were detected prior to significant loss of cell viability, indicating that disruption of heme metabolism represents an early event rather than a secondary consequence of cell death. The mixture induced marked and sustained PPIX reduction from 18 h onward. Mixture coefficient modeling, capturing both individual and interaction effects, revealed a time-dependent shift in analogue contribution, with ENNB₁ emerging as the dominant driver from 30 h onward and interaction effects strengthening at later time points. Transcriptomic analysis revealed regulation of genes associated with heme biosynthesis, oxidative stress, apoptosis, and ferroptosis-related pathways.

These findings suggest that enniatins impair hepatic cell viability through early interference with heme metabolism, potentially causing iron dysregulation and ferroptosis. Considering the frequent co-occurrence of ENNs in aquafeeds, combined exposure may pose a greater toxicological risk than individual compounds, underscoring the need for further mechanistic studies and improved risk assessment in aquaculture.

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2.31 L31 - Heme biosynthesis under mycotoxin pressure in Atlantic salmon: a multi-target investigation

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Plant-derived ingredients in salmon feed, mainly cereals and by-products, increased farmed fish exposure to cereal-associated mycotoxins, including frequently occurring but still poorly regulated *Fusarium* metabolites, such as enniatin B (ENNB) and beauvericin (BEA) (1). These have been associated with adverse effects in Atlantic salmon, including growth impairment and anemia-like outcomes (2). However, the molecular events linking dietary exposure to these adverse phenotypes are yet to be unveiled, limiting mechanism-driven hazard interpretation and prioritization. A plausible explanation involves heme metabolism. Heme availability is central to oxygen transport, mitochondrial respiration, and redox balance; consequently, perturbations in heme production can propagate into oxidative stress, mitochondrial dysfunction, and altered iron handling.

Here, we evaluated whether ENNB, its congener ENNB1, and BEA can possibly interact with representative (Atlantic salmon) proteins involved in heme biosynthesis.

We applied a cutting-edge computational pipeline based on Boltz-2 (3), an AI-based 3D modelling tool, followed by molecular dynamics simulations (300 ns each, 2 independent replicates) and free energy computation, to assess binding plausibility and stability over time. We focused on a multi-target panel covering key nodes of the heme biosynthetic pathway, including HMBS, UROS, UROD, CPOX, PPOX, and FECH. The analysis is being replicated also on the orthologue's human targets.

The analysis supported potentially stable interactions for the tested compounds across a subset of the *in silico* tested targets, providing a set of target protein-mycotoxin candidates that could impair heme biosynthesis and thereby cause redox dysfunction and iron mediated ferroptosis consistent with anemia-like outcomes. Some of the most promising candidates are getting thoroughly evaluated by targeted *in vitro* testing using salmonid-model systems, to validate the predicted computational interactions and clarify their functional consequences. Overall, this pipeline sets the ground and supports a mechanism-driven interpretation of emerging mycotoxin risks in modern Atlantic salmon aquafeeds.

This study falls within the MYTOXA project (Norwegian Research Council, grant number 34401), integrating *in vivo*, *in vitro*, and *in silico* approaches to assess how feedborne exposure to emerging mycotoxins influences salmon growth and smoltification during the freshwater phase.

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2.32 L32 - How the mycotoxin deoxynivalenol exacerbates the genotoxicity of heme iron

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Humans are constantly exposed to a complex mixture of food contaminants, a concept known as the "chemical food exposome". Among natural contaminants, mycotoxins are the most prevalent. Deoxynivalenol (DON) affects nearly half of all cereal crops, a figure rising due to climate change, and currently exposes about 80% of the population. While DON is not itself classified as a carcinogen (1), it is known to exacerbate DNA damage caused by other agents (2,3,4). We hypothesized that DON might similarly amplify the genotoxic and carcinogenic potential of heme iron, found in red and processed meats. Its consumption is associated with colorectal cancer through a mechanism involving the production of cytotoxic aldehydes in the gut (5).

In vitro experiments on intestinal epithelial cells confirmed that heme iron causes DNA damage, as evidenced by DNA strand breaks detected by the comet assay and H2AX Ser139 phosphorylation labeling. Using non cytotoxic doses of DON, the results showed that DON alone is not genotoxic whereas co-exposure significantly increased the genotoxic effects of heme iron. This combined exposure also heightened genomic instability with an increase of micronuclei frequency in the co-exposure condition, and promoted phenotypic cell transformation indicated by an increase in colony growth in soft agar assays. Transcriptomic profiling indicates that the mixture exhibits characteristics of both compounds alone and suggests the involvement of oxidative stress and inflammatory responses.

These findings were validated *in vivo* using a rat model fed for four weeks with a diet containing both DON and heme iron. Analysis of colon tissue revealed that the combined treatment led to higher levels of genotoxicity compared to agents administered alone. Furthermore, markers of rapid epithelial turnover such as increased mitotic activity and crypt fission, representing early alterations often associated with an increased risk of polyp formation.

Taken together, these results demonstrate that DON exacerbates the adverse intestinal effects of heme iron, specifically amplifying genotoxicity, genomic instability, and cell transformation, three key alterations involved in carcinogenesis. Thus, the joint presence of DON and heme iron in the diet warrants consideration in foodborne health risk evaluations.

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2.33 L33 - Deoxynivalenol disrupts the blood-testis barrier in prepubertal and adult mice: a possible cytokine-mediated effect

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Deoxynivalenol (DON) is one of the mycotoxins with the greatest economic impact, and evidence has demonstrated its deleterious effects on male reproductive system (1). DON is known to increase the permeability of the blood–testis barrier (BTB), accompanied by reduced expression of junctional proteins (2). Considering that cytokines play an essential role in the restructuring of cell junctions during spermatogenesis (3), the aim of this study was to quantify the levels of TNF- α and IL-1 β and evaluate the morphology of seminiferous tubules in prepubertal and adult Swiss mice exposed to DON (CEUA nº 27/2019). Thirty prepubertal (21 days old) and thirty adult (65 days old) mice were fed either a control diet or a diet containing 10 mg DON/kg feed for 15 days (prepubertal) or 28 days (adults). The testes were subjected to TNF- α and IL-1 β quantification by ELISA and morphological evaluation. The data were checked for normality and homogeneity and analyzed using Student's t-test or the Mann-Whitney test ($p < 0.05$). Prepubertal mice exposed to DON showed a significant increase in IL-1 β (CTRL: 28.24 ± 4.14 ; DON: 44.21 ± 16.71), while in adults a significant elevation of TNF- α was observed (CTRL: $19.71 [13.97–20.42]$; DON: $33.83 [24.30–49.38]$). DON also resulted in a higher proportion of abnormal seminiferous tubules in both pre-pubertal (CTRL: 1.5 ± 0.43 ; DON: 4.8 ± 0.80) and adult (CTRL: 4.8 ± 0.92 ; DON: 9.8 ± 2.11). The main alterations were epithelial vacuolization and the presence of somatic cells in the tubular lumen. DON compromises BHB by reducing junctional proteins such as occludin, N-cadherin, and claudin-11. However, the maintenance of the BHB depends on the coordinated action of cytokines, which during spermatogenesis allows the translocation of germ cells in the seminiferous epithelium (3). The observed increases in TNF- α and IL-1 β may therefore contribute to DON-induced BTB dysfunction, including in prepubertal individuals, a developmental stage that remains poorly explored. The morphological alterations identified reinforce the hypothesis of BHB dispairment and suggest deleterious effects to reproduction. Although these findings indicate the participation of inflammatory pathways in the toxic mechanism of DON, further studies are needed to clarify its long-term consequences.

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2.34 L34 - Shaping the immunomodulation in colon: how high glucose conditions modulate the immunomodulatory properties of mycotoxins

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Mycotoxins are widespread food contaminants, reported to modulate the immune response, influencing mucosal homeostasis and local inflammation in colon. Deoxynivalenol (DON), a trichothecene mycotoxin commonly contaminating cereal-based products, is known from its immunomodulatory properties. Although its inflammatory effects in the gut have been characterized, considerably less is known about how the metabolic microenvironment in colon influence DON- driven effects. In the context of the increasing prevalence of hyperglycemia and metabolic disorders, it is critical to determine how elevated glucose levels influence intestinal immune responses to mycotoxin exposure.

In this study we investigated how high glucose conditions modulate the immunomodulatory activity of DON in *in vitro* model of colon enterocytes. We assessed cytokine production and inflammatory signaling pathways under the normoglycemic and hyperglycemic conditions as well as inflammatory environment. The hypothesis was tested with different molecular biology methods: RTqPCR, Western blot, ELISA and confocal microscopy. Our findings indicate that elevated glucose levels significantly alters mycotoxins-induced immune signaling, affecting pro-inflammatory cytokine expression and key regulatory pathways associated with immune activation in colon cells.

The results suggest that metabolic context is an important determinant of DON-induced immunomodulatory effect in the colon. High glucose conditions, common in the population, might alter the DON-induced immune responses, potentially influencing the inflammatory outcome in the colon. This work highlights the importance of integrating the metabolic parameters into toxicological risk assessment and provides new insight into the host-environment interactions at the intestinal barrier, shaping the real scenario of mycotoxin exposure.

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2.35 L35 - Unraveling the toxicokinetics of T-2 and HT-2 toxin in humans and developing a physiologically-based kinetic model

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The absorption, distribution, metabolism and excretion patterns of the trichothecene mycotoxins T-2 toxin (T-2) and HT-2 toxin (HT-2) in humans are majorly unknown. This limits our understanding of internal exposure to these compounds, and hinders the application and interpretation of human biomonitoring. T-2 and HT-2 are commonly detected in cereals and cereal-based products, widely consumed in Europe, therefore, robust assessment of their exposure is important.

Our research aimed to further characterise the toxicokinetics of T-2 and HT-2 and to develop a physiologically based kinetic (PBK) model, enabling prediction of urinary biomarker concentrations based on external exposure and *vice versa*. Urine voids across 24 h from 40 Norwegian adults were analysed for T-2 and its metabolites using targeted and suspect screening analytical approaches. Dietary exposure of these participants was estimated using 24-h weighed food records combined with Norwegian occurrence data. In parallel, toxicokinetic information on the mycotoxins was generated using *in vitro* human liver microsome experiments and a literature review.

Total HT-2 (mycotoxin and its glucuronides) was detected in 97% of the 24-h urine voids. Total T-2 and T-2 triol were not detected. Suspect screening tentatively identified the following mono- and di-hydroxylated metabolites of T-2 and HT-2. In our *in vitro* liver microsome experiments, 98% of T-2 was metabolised within 2 h, with HT-2 accounting for 97% of the metabolites. Following a 2 h incubation with HT-2, 73% of the added HT-2 remained unchanged, and a large fraction was unidentified compounds (26%).

This multifaceted research advances the understanding of the human toxicokinetics of T-2 and HT-2, and demonstrates the utility of PBK modelling to link external and internal exposure. The identification of multiple hydroxylated urinary metabolites suggests that HT-2 alone does not fully represent internal exposure. These results were used to inform the design of a controlled toxicokinetic study in a group of adults which is currently underway, where we aim to further explore the toxicokinetics of T-2 following a known dose.

2.36 L36 - Beyond aflatoxin B₁: Mutagenicity assessment and evaluation of topoisomerase-poisoning potential of selected aflatoxin B₁ precursors

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Mycotoxins are naturally occurring food contaminants capable of exerting severe adverse effects on human health. Among them, aflatoxins are of particular concern, with aflatoxin B₁ (AFB₁) being the most potent naturally occurring human carcinogen, primarily targeting the liver. Hence, its presence in food is strictly regulated in the European Union. The biosynthesis of AFB₁ involves multiple enzymatic reactions that generate a variety of intermediates, collectively referred to as aflatoxin B₁ precursors. As the yields of these enzymatic reactions are not 100 %, these precursors occur as co-contaminants alongside aflatoxins in food. In contrast to AFB₁, no maximum levels have been established for these precursors due to the lack of toxicological and occurrence data.

The present study aimed to address this knowledge gap by investigating the mutagenic properties and the topoisomerase-poisoning potential of selected AFB₁ precursors (i.e. averantin (AVN), averufin (AVF), versicolorin A (VerA), *O*-methyl-sterigmatocystin (OMST), and sterigmatocystin (STC)). Mutagenicity was assessed using the Ames test with *Salmonella typhimurium* strains TA98 and TA100, enabling the detection of frameshift mutations and base-pair substitutions, respectively. In addition, the hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutagenicity test was carried out in V79 cells (chinese hamster fibroblasts) for AVN, which showed ambiguous results in the Ames test. Both assays were executed in the presence and absence of rat S9 fraction to elucidate the role of metabolism on the mutagenic properties of the test compounds. To further investigate topoisomerase-poisoning activity by precursors containing an anthraquinone moiety, the *in vivo* complex of enzyme (ICE) assay was implemented using A-431 cells (human epidermoid carcinoma cell line).

Results of the Ames test demonstrated that VerA, OMST and STC induced both frameshift mutations and base-pair substitutions in a concentration-dependent manner upon metabolic activation. In contrast, AVN exhibited mutagenic effects only in the absence of S9 mix, while AVF did not show mutagenic potential under any of the tested condition. The HPRT test indicated that AVN did not induce mutations of the HPRT gene, regardless of metabolic activation. Results of the ICE assay displayed the ability of AVF and VerA to poison both topoisomerase II α and II β at the highest concentration tested (25 μ M).

In conclusion, the results of this study provide first insights into the mutagenic potential of several AFB₁ precursors, showing that all compounds (except AVF) exhibited mutagenic activity, while VerA and AVF acted as topoisomerase poisons targeting topoisomerase II. These findings underscore the need for further research on these poorly described compounds, while also serving as an important foundation for a future, comprehensive toxicological evaluation.

Acknowledgments

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2.37 L37 - Acute Toxic Effects of *Stachybotrys chartarum* Bioaerosols

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Dampness-related indoor mold is a global health problem, with *Stachybotrys chartarum* being particularly associated with respiratory diseases. However, causal exposure-response relationships are still lacking. The aim of our research project was therefore to develop an in-vitro test system to evaluate the inhalation-toxic potential of mold bioaerosols using lung epithelial cells (NuLi-1) and macrophages (THP-1).

We inoculated common indoor building materials (plasterboard, woodchip wallpaper) and ME-agar as reference with *S. chartarum* CBS, *S. chartarum* WT, *Aspergillus versicolor*, *Penicillium chrysogenum*, and *Alternaria botrytis* (4-8 weeks growth, 25°C/90% RH). Extracts from whole materials and surface swabs were prepared and exposed to the cells. Cell viability, pro-inflammatory cytokines (GM-CSF/IL-1 β via ELISA), and cell impedance (ECIS[®]) were analyzed. Mycotoxin composition and concentration analysis were also done.

S. chartarum CBS exhibited maximum cytotoxicity with 0-10% cell viability in both cell lines, complete GM-CSF inhibition, and strong IL-1 β induction. Aerosol extracts from woodchip wallpaper caused 35.8% (NuLi-1) and 0.6% (THP-1) viability. There were clear material-dependent differences: cellulose-containing materials (plasterboard, woodchip wallpaper) were significantly more toxic than ME-agar. Mycotoxin composition and concentration analysis confirmed elevated levels of the mycotoxins Roridin L2, Verrucrin J, and Stachybotrylactam.

Conclusion: The test system developed shows acute toxic effects of *S. chartarum* CBS bioaerosols with ribosome-inhibiting mechanism (Trichothecene Type D). It enables species- and substrate-specific risk assessment and highlights health risks in damp environments.

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2.38 L38 - Hydrothermal treatment with sodium metabisulfite of deoxynivalenol contaminated maize as effective tool of inactivation

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The treatment of deoxynivalenol (DON) contaminated grain with sodium metabisulfite (SBS) is known to inactivate DON to the less toxic metabolites DON sulfonate (DONS)1, DONS2 and DONS3. So far, the treatment was mainly investigated as wet preservation technique, resulting in feed material not suitable for trading. Recently a hydrothermal treatment of grain with SBS was optimized overcoming those limitations (1). However, the effectiveness of this treatment still needs to be validated biologically i.e. by the reduction of the internal dose of animals fed such treated material.

Two maize batches, a control maize (CON) and a *Fusarium* contaminated maize (FUS) mainly containing DON (3.94 mg/kg) were treated with or without 5 g/kg SBS (+/-) in a stationary experimental conditioner (Type RF72W/RK3, Süddeutsche Elektromotoren – Werke, Bruchsal, Germany) with a saturated steam addition of 3 % for 10 seconds. The treated maize was included in experimental diets for piglets at 50 %. The resulting 4 groups (CON-, CON+, FUS-, FUS+) were tested in a feeding trial with 80 piglets, 20 animals per group. Piglets were housed in floor pens with 4 piglets per pen and 5 pens per treatment. The experiment started at a mean live weight of 9.2 ± 1.2 kg and lasted 5 weeks with *ad libitum* excess to feed and water. Before the start and at the end of the experiment blood samples were taken by venipuncture. Feed not consumed and animals were weighed every week. Feed and blood serum samples were analysed for DON, DONS1, DONS2 and DONS3 by LC-MS/MS.

The concentration of DON in the experimental diets was reduced by 60 and 67 % in CON+ and FUS+ diets compared to CON- and FUS- diets, respectively. At the same time, DONS2 and DONS3 were detected in the diets including the SBS treated maize. Performance of piglets with a mean cumulative weight gain of 658 g/d was high and not influenced by the contamination of the diets or the maize treatment. Similarly, feed intake was not affected by the experimental treatment while feed efficacy was slightly improved for the *Fusarium* contaminated maize ($p_{\text{maize}}=0.007$). The serum concentrations of DON reflected the reduction of the concentrations in the feed with 0.84 ng/ml; 0.57 ng/ml; 6.22 and 3.48 ng/ml for piglets of group CON-, CON+, FUS- and FUS+, respectively. Additionally, DONS2 was detected in the serum of the piglets receiving the diets including the SBS treated maize.

Therefore, the hydrothermal treatment with SBS of DON contaminated maize proved to be effective in reducing the internal exposure of piglets fed diets including the treated grains and therefore successfully inactivated DON.

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2.39 L39 - Characterization of a detoxified deoxynivalenol metabolite supporting microbial mitigation strategies in food safety

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Mycotoxins commonly contaminate food and feed products (1). Among them, Deoxynivalenol (DON) is a prevalent trichothecene mycotoxin that exerts its toxicity through ribosome binding and induction of ribotoxic stress, posing significant risks to human and animal health (2). Microbial detoxification through enzymatic biotransformation offers a promising approach to mitigate DON toxicity (3). Deoxynivalenol-8,15-hemiketal-7-glucoside (HKDON7G), a glycosylated DON metabolite generated by the glycosyltransferase YjiC, has been identified as a potential detoxification product (4), yet its biological activity remains largely uncharacterized. To address this gap, the present study provides a comprehensive comparison of DON and HKDON7G toxicity using *in vitro* intestinal epithelial cell models and *ex vivo* porcine jejunal explants, a highly relevant model for human intestinal physiology (5), complemented by mechanistic analyses of ribosome interaction and downstream signaling.

In contrast to DON, HKDON7G exhibited no cytotoxicity on proliferative cells or barrier-disruptive effects on differentiated monolayers, and did not modify tight junction protein abundance. On intestinal explants, HKDON7G failed to provoke histopathological alteration and inflammatory responses. Genome-wide expression profiling of intestinal tissue demonstrated that HKDON7G had no effect on the transcriptome of exposed cells, even at elevated concentrations, further supporting its lack of toxicity. Molecular modeling demonstrated that the glucose moiety of HKDON7G imposes steric hindrance that precludes its accommodation within the ribosomal active site. Consistently, HKDON7G neither inhibited protein synthesis nor activated the p38 or SAPK/JNK MAP kinase pathways, suggesting that it does not trigger a ribotoxic stress response.

Taken together, these results provide strong mechanistic evidence supporting the safety of HKDON7G and underscore the potential of microbial-based detoxification strategies to improve food and feed safety.

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2.40 L40 - Impact of UV-C treatment on *Alternaria* spp. growth and *Alternaria* mycotoxins *in vitro* and in tomato

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Species of the genus *Alternaria* are common phytopathogens of tomato fruit (*Solanum lycopersicum*) and are known producers of toxic secondary metabolites such as alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TeA). These mycotoxins may persist during processing, representing a potential food safety concern. Physical decontamination strategies, including UV-C radiation, have been investigated as non-thermal alternatives to control fungal growth and reduce mycotoxin contamination. Data on the impact of UV-C treatment on *Alternaria* growth and toxin behaviour in tomato-derived matrices remain limited. The aim of this study was to evaluate the effect of UV-C radiation on *Alternaria* growth *in vitro* and in tomatoes, and to preliminarily assess its effect on *Alternaria* toxin levels in tomato, tomato purée and juice.

The effect of UV-C radiation on *Alternaria* growth was evaluated using an *in vitro* assay on Potato Carrot Agar and on artificially inoculated tomatoes. UV-C treatments were applied at doses of 0, 5, 10, and 20 kJ/m². For processed matrices, tomato juice and tomato purée were subjected to the same UV-C doses. To assess toxin behaviour independently of fungal growth, tomato juice and tomato purée were spiked with AOH, alternariol AME, and TeA at concentrations of 0, 50, 100, and 200 ppb prior to UV-C treatment in triplicate. Untreated samples (0 kJ/m²) were used as controls. Fungal growth inhibition and toxin levels were evaluated after treatment. Mycotoxin levels were determined using an in-house validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) method with matrix-matched calibration curves.

UV-C radiation showed an inhibitory effect on *Alternaria* growth *in vitro* and on tomato fruit, with increased inhibition observed at higher doses. Preliminary observations also indicate an effect of UV-C treatment on toxin levels in tomato juice and purée, both in naturally contaminated and spiked samples.

2.41 L41 - From the Plate to the Plant: Discovering the biocontrol potential of *Hanseniaspora uvarum* against *Aspergillus flavus*

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Aspergillus flavus is a saprophytic and ubiquitous soil fungus with the potential to contaminate maize, tree nuts and other crops, and is a primary producer of the carcinogenic mycotoxin aflatoxin B₁ (AFB₁). Several strategies have been implemented to prevent the contamination of food and feed by AFB₁ in pre-harvest field conditions. Among the most successful strategies is the application of biocontrol agents.

Hanseniaspora uvarum is a yeast commonly isolated from fruit surfaces that has previously demonstrated biocontrol potential against several mycotoxigenic fungi including *A. flavus* [1]. The aim of this work was to study the ability of *H. uvarum* U1 to control *A. flavus* growth and AFB₁ production using both *in vitro* and *in planta* approaches and to identify potential mechanisms of action that contribute to its effectiveness.

Our results indicate a 20% and 95% reduction in *A. flavus* growth and AFB₁ production, respectively, when treated with *H. uvarum* U1 on Czapek-Yeast (CYA) medium. Further, the production of chitinases, siderophores, or volatile organic compounds (VOCs) were tested as possible mechanisms involved in its biocontrol action. To examine the production of VOCs, we used the double-dish system (DDS) described by Ruiz-Moyano [2], VOCs were extracted from the DDS system by solid-phase microextraction and analyzed by GC/MS. Among the identified volatiles, Ethylene Glycol Diacetate (EDT) was tested against *A. flavus* on CYA medium at concentrations of 357, 715, and 1430 µL/L of air volume (headspace) within the double-dish system. In this assay, a significant decrease in both *A. flavus* growth (15–25%) and AFB₁ presence (80–95%) was observed with two of the tested EDT concentrations (357 and 1430 µL/L).

To examine the effectiveness of *H. uvarum* U1 biocontrol *in planta*, maize kernels were either pretreated with the yeast followed by *A. flavus* inoculation 48 hours later, or both microorganisms were co-inoculated simultaneously. In both cases, maize kernels were inoculated via kernel pricking with *A. flavus* 10 days after pollination and harvested 7 days after inoculation. In both strategies, AFB₁ was significantly reduced by 90% (pretreatment) and 73% (co-inoculation) while the *A. flavus* growth remained unaffected by the yeast treatment maintaining the same levels as the control cobs. Overall, these findings demonstrate that *H. uvarum* U1 has potential as a biocontrol agent against *A. flavus* contamination in crops such as maize and mechanistically could be due to the production of VOCs.

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2.42 L42 - Transcriptomic profiling of aflatoxin B1 exposed and medicinal herb supplemented pig liver

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Aflatoxin B1 (AFB1) is a toxic secondary metabolite produced by *Aspergillus* fungi. Dietary exposure of AFB1 strongly induces adverse effects in farm animals. EU feed legislation sets maximum AFB1 at 20 µg/kg in pig compound feed (piglets: 5 µg/kg; 12% moisture) [1]. With rising climate-driven contamination risk and non-exhaustive monitoring, especially for on-farm feed, contaminated lots can still enter rations and exceed legal limits [2]. Chronic exposure of AFB1 in feed can lead to critical physiological and pathological consequences in animals, including reduced growth rates, reproductive issues, immunosuppression, and gut barrier dysfunctions. Since the complete eradication of AFB1 from feedstock through physical and chemical methods is ineffective, several natural additives with anti-toxic properties have been widely investigated as potential AFB1 mitigation strategies. Extracts from medicinal herbs and their constituent bioactive compounds have demonstrated significant hepatoprotective activity, aiding in the restoration of liver integrity [3]. Accordingly, a nutritional experiment was performed incorporating AFB1 (120 µg/kg BW) exposure via feed; kalmegh (*Andrographis paniculata*: 30 mg/kg BW), milk thistle (*Silybum marianum*: 90 mg/kg BW) and turmeric (*Curcuma longa*: 90 mg/kg BW) supplementation (BW: body weight) in distinct pig groups, followed by RNA sequencing of liver tissue (n = 44). Serum biochemical parameters associated with liver health were analyzed to compliment the gene expression data. The analysis of differentially expressed genes (DEG) in AFB1-exposed animals revealed transcriptomic modulations in genes like *CYP2A19*, *CYP1A2*, *GSS*, *GPT2*, and *PCK1* related to AFB1 primary biotransformation, mounting of toxic responses and reduced energy derivation. Alternatively, with medicinal herbs, a positive adaptive regulation of respective genes *CYP27A1*, *ACSS2*, and *HAMP* was observed in terms of metabolic activation, energy and iron homeostasis. Furthermore, AFB1 exposure in pigs resulted in modulated serum hepatic parameters suggesting an onset of aflatoxicosis. Conversely, in medicinal herb supplemented animals, the absence of significant differences in biochemical parameters levels indicated an unaffected healthy liver function. These findings provide a foundation for delineating the specific molecular mechanisms related to both AFB1 exposure and medicinal herb supplementation and account for possible nutritional strategies in future aimed at mitigating AFB1 toxicity in animals using natural additives.

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3 Poster Abstracts

3.1 P1 - Species-level *Fusarium* resolution in cereals: a TEF1 metataxonomic approach

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Cereal safety is a critical public health concern, as crops are frequently contaminated by mycotoxin-producing fungi, particularly from the genus *Fusarium*. The toxigenic potential of *Fusarium*, including the production of trichothecenes (T-2, HT-2, DON), zearalenone, and fumonisins, is highly species-dependent. Although metataxonomics is a powerful tool for characterizing fungal communities, the universal ITS2 marker lacks the resolution required to distinguish closely related species with distinct toxigenic profiles (1). This work proposes a high-resolution metataxonomic workflow within the QIIME 2 environment to optimize *Fusarium* identification using the translation elongation factor 1- α (TEF1) gene. We developed a TEF1 Naive Bayes classifier trained on a curated database derived from FUSARIUM-ID v.3.0 (2), expanded with eukaryotic sequences to prevent false-positive assignments. To validate this approach, a total of 30 samples of small-grain cereals (12 of wheat, 11 of barley, and 7 of oat) were collected from 11 Spanish cereal-producing regions. After DNA extraction, ITS2 and TEF1 libraries were sequenced on a paired-end Illumina platform. Sequences were analyzed to provide a direct comparison of TEF1 and ITS2 across grain samples. Our findings demonstrate that ITS2 significantly underestimates *Fusarium* diversity, whereas TEF1 enables precise species-level resolution. *Fusarium* ITS2 ASVs were predominantly assigned to *F. sporotrichioides*, *F. tricinctum*, or remained unresolved as *Fusarium* sp. Conversely, TEF1 analysis of the same samples revealed a much more diverse taxonomic profile within the *F. tricinctum*, *F. sambucinum*, and *F. incarnatum-equiseti* species complexes, and a sharp reduction in the relative abundance of unassigned *Fusarium* spp. This approach confirmed the persistence of key toxigenic species in all cereals tested, such as *F. langsethiae*, and revealed a wider toxigenic potential through the consistent detection of species often overlooked by traditional methods, including *F. equiseti*, *F. acuminatum*, and *F. culmorum*. Although ITS2 failed to resolve sequences at the species level, it provided an overall characterization of the mycobiota. We conclude that the combination of high-resolution markers like TEF1 with broader markers like ITS2 is essential for accurate health risk assessments involving *Fusarium* toxins.

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3.2 P2 - Exploring Toxic Interactions Between Aflatoxin B1 and Its Precursors

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Aflatoxin B1 (AFB1) is a mycotoxin primarily produced by *Aspergillus flavi* and *A. parasiticus* mainly known to be highly carcinogenic to humans (Group 1, IARC). Its biosynthetic precursors, versicolorin A (VerA) and sterigmatocystin (STC), also exhibit significant genotoxic properties. STC is classified as possibly carcinogenic to humans, with limited evidence in humans and insufficient evidence in experimental animals (Group 2B, IARC), whereas VerA has been shown to induce genotoxic and cytotoxic effects in various cellular models.

The co-occurrence of these mycotoxins in contaminated food products represents a major concern. Aflatoxigenic fungi produce all these three compounds, and food and feed commodities may be contaminated by both aflatoxigenic fungi (producing AFB1) and non-aflatoxigenic species such as *A. nidulans* and *A. versicolor*, which produce STC and VerA. Moreover, human diets typically consist of diverse food commodities, increasing the likelihood of simultaneous exposure to multiple mycotoxins. The combined action of toxins can be additive (effects simply sum), synergistic (combined toxicity exceeds the sum), or antagonistic (combined toxicity is reduced because one toxin counteracts the other).

This study investigated the combined genotoxic effects of AFB1, VerA, and STC to better assess their potential impact on human health.

Genotoxicity was assessed by measuring γ H2AX expression, a marker of DNA double-strand breaks, using an In-Cell Western assay under predefined non-cytotoxic concentrations using the non-cancerous human colonic epithelial cell line HCEC-1CT. Dose-dependent responses were obtained for all mycotoxins alone or in combination for short and prolonged periods, followed by a recovery phase to assess potential delayed or persistent effects. Toxin ratios were derived from occurrence data published by the European Food Safety Authority (EFSA), and interactions were calculated using a component-based approach.

The results demonstrated that genotoxic responses were strongly dependent on exposure duration and recovery time. While DNA damage was observed directly after short-term exposure DNA damage was detected after a recovery period, with STC and VerA exhibiting higher genotoxic potency than AFB1. All three toxins induced comparable levels of DNA damage after prolonged exposure regardless of recovery. Mixture toxicity analysis revealed additive effects for equipotent combinations regardless of the exposure conditions. In contrast, in the case of mixtures representing occurrence-based ratios we observed synergistic genotoxic interactions in mixtures containing VerA.

Overall, this study provides important insights into the interactions between AFB1 and its biosynthetic precursors, highlighting the need for a deeper understanding of their combined effects. Such knowledge is essential for improving food safety risk assessment and reducing health risks associated with mycotoxin contamination in food products.

3.3 P3 - Long-Term Monitoring of Mycotoxin Occurrence in Czech Malting Barley under Climate Variability

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Mycotoxins are toxic secondary metabolites produced by microscopic filamentous fungi. Among the most important with regard to human health are aflatoxins, ochratoxin A, trichothecenes, and zearalenone. Contamination of malting barley with these compounds poses a risk to raw material safety as well as to the malting and brewing industry. Fungal growth and mycotoxin production are strongly influenced by temperature and moisture; therefore, climate variability may significantly affect the level of contamination in individual harvest years.

At the Research Institute of Brewing and Malting in Brno, contamination of malting barley in the Czech Republic has been systematically monitored since 2008 (1–4). The occurrence of legislatively regulated mycotoxins is evaluated in individual harvest years, enabling the assessment of long-term trends, year-to-year variability, and the relationship between climatic conditions and contamination levels.

This work was supported by the Ministry of Agriculture of the Czech Republic under Institutional Support RO1923.

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3.4 P4 - Hybrid *in vitro*/*in silico* approach to elucidate the effect of mycoestrogens on barrier integrity via tight junction protein claudin-4

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Endocrine-disrupting mycotoxins such as alternariol (AOH) and zearalenone (ZEN) can interfere with hormonal signaling pathways, thereby contributing to metabolic imbalance [1, 2]. Yet their capacity to modulate intestinal barrier integrity has remained poorly defined. In this study, a new approach methodology (NAM)-based hybrid workflow combining advanced *in vitro* assays with *in silico* modeling was established to investigate the effects of both mycotoxins on the human gut epithelium. Using a differentiated Caco-2/HT29-MTX-E12 co-culture that recapitulates essential features of the intestinal barrier [3], exposure to AOH and ZEN caused reproducible alterations in claudin-4 distribution along the apical junctional axis, paralleled by changes in nuclear morphology and cellular organization within the monolayer.

Molecular docking analyses [4] predicted stable interactions of both mycotoxins with claudin-4, displaying higher docking scores than endogenous steroidal ligands. Subsequent molecular dynamics simulations [5] revealed that these interactions could perturb the stability of residues lining the paracellular cleft and induce subtle, membrane-composition-dependent shifts in root-mean-square deviation and fluctuation, suggesting a local dynamic destabilization of tight junction architecture.

The combined *in vitro*/*in silico* approach introduced here establishes a versatile framework for probing the molecular basis of xenobiotic-induced epithelial barrier disruption.

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3.5 P5 - Impact of maize–bean intercropping on crop growth and Fusarium mycotoxin contamination

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Seed- and soil-borne pathogens of the genera *Fusarium*, *Rhizoctonia*, and *Pythium* that infect maize plants during the seedling stage pose a threat to optimal seedling emergence, plant development and yield stability. Additionally, *Fusarium* infections may result in the accumulation of mycotoxins in the harvested material. To mitigate the risk of infections, diversified crop rotations and integrated pest management strategies are often recommended. Maize-bean intercropping is known to improve biodiversity and pole beans have proven effective because they fix nitrogen for the maize plants. The maize-pole bean silage is used as feed for cattle and pigs as higher crude protein levels are achieved compared to pure maize silage. The extent to which *Fusarium* infection of the seeds may affect the growth and yield of the maize-bean mixture, and whether it leads to an accumulation of mycotoxins in the silage maize, are the subjects of our investigations. Therefore, we cultivated maize bean mixtures on two field sites in Braunschweig. Seeds were either non- treated (control), or infected with *F. culmorum* spores. In addition, maize was grown alone. For all field trials, four plots per mixture and treatment were sown in a randomized block design. Emergence and early development of the plants were recorded at growth stages BBCH14 and BBCH32. Finally, silage maize was harvested at the respective maturity stages of the plants to collect yield data. Subsamples were further analyzed for *Fusarium* toxin contamination. In detail, we will provide data on zearalenone, deoxynivalenol and fumonisin contents in relation to the mixtures and seed treatments.

3.6 P6 - Sulfation as a detoxifying mechanism for the estrogenicity of the mycotoxin alternariol

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The foodborne mycotoxin alternariol (AOH) has been associated with several adverse effects, including cytotoxic, genotoxic, mutagenic, immunosuppressive, and hormone-mimicking properties. However, AOH is known to undergo extensive metabolism in mammalian systems, providing a rationale to evaluate how biotransformation influences its toxicological potential.

Earlier studies have shown that sulfation and glucuronidation are the major conjugation pathways of AOH, the latter markedly reducing the DNA-strand-breaking activity of the parent compound in HT-29 cells (1). Conversely, an *in-silico* study predicted that alternariol-3-*O*-sulfate (AOH-3-S) exhibits a binding affinity to estrogen receptor α (ER α) comparable to that of AOH (2). This finding calls for a re-evaluation of the toxicological relevance of AOH-3-S – a metabolite not only arising from phase II metabolism in humans but also occurring as a masked mycotoxin in foodstuffs (3).

To address this, the estrogen-sensitive cell line Ishikawa was incubated with various concentrations of AOH-3-S, both alone and in combination with 17 β -estradiol (E2) to assess pro- or anti-estrogenic potential. These were quantified using the alkaline phosphatase (AIP) assay, which revealed an absence of significant estrogenic activity for AOH-3-S in contrast to the parent compound. Cell viability, assessed by the CellTiterBlue™ assay, remained unaffected, thereby excluding cytotoxicity as a causative agent for lacking AIP induction. Consequently, it needed to be evaluated whether poor cellular uptake or the inability of estrogen receptor binding might be responsible for the absence of estrogenicity. The AOH and AOH-3-S concentration in Ishikawa cell supernatants and lysates were analyzed *via* LC-MS/MS. Upon exposure to AOH-3-S, neither the conjugate nor its parent compound was detected intracellularly in considerable amounts. In contrast, direct treatment with AOH resulted in efficient cellular uptake and the formation of two sulfate metabolites in low concentrations.

Overall, our findings suggest that AOH-3-S exhibits lower toxicity than AOH in the investigated cell line, presumably because its elevated polarity limits cellular uptake. However, further studies are required to determine whether AOH-3-S formed intracellularly through metabolic conversion can exert estrogenic or other ER α -mediated effects, such as apoptosis. Overall, this study provides valuable insights into the effects of metabolism on the toxicological profile of AOH and contributes to the broader understanding of masked mycotoxins.

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3.7 P7 - Reduction of polar mycotoxins in malting steeping water using the ACMalt recycling technology

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Malting is associated with high potable water consumption, with approximately 5 m³ of water used per tonne of barley during steeping, depending on the applied technology. To reduce water demand, some maltings reuse the same steeping water in multiple cycles. However, this practice may lead to the gradual accumulation of organic compounds, germination inhibitors, and contaminants released from barley surfaces (1). Among these compounds are polar mycotoxins produced by *Fusarium* species, particularly deoxynivalenol (DON) and other can transfer from grain into steeping water (2), as well as pesticide residues originating from agricultural treatments. Repeated water reuse may therefore promote contaminant accumulation and affect both process safety and technological performance. The use of fresh potable water represents an important decontamination step contributing to the reduction of such substances (3).

The aim of this study was to evaluate the ability of the multistage steeping water recycling technology ACMalt to reduce concentrations of polar mycotoxins and pesticide residues and enable safe water reuse without their accumulation. The technology has been installed in a traditional floor malting facility in Záhlinice (Czech Republic), producing approximately 2,100 tonnes of malt annually and consuming around 14,000 m³ of water per year. The ACMalt system combines biological treatment in a CSTR bioreactor with activated sludge, membrane ultrafiltration, and a final adsorption step based on a carbon nano-adsorbent. Steeping water samples were analysed by LC-MS/MS before and after treatment. Initial analyses confirmed the presence of mycotoxins and pesticide residues in untreated water. After application of the technology, a significant reduction of the monitored compounds was observed, with several analytes reduced below the limit of quantification.

The results demonstrate that targeted water treatment can effectively limit the accumulation of water-soluble mycotoxins and pesticide residues during steeping water reuse and support safe and sustainable water management strategies in maltings.

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3.8 P8 - Mycotoxin analysis using strip tests and chromatographic methods

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Food and feed safety remains a major global concern, prompting the development of extensive regulatory frameworks, particularly within the European Union, to control contaminants such as mycotoxins. These toxic secondary metabolites, produced mainly by moulds of the genera *Aspergillus*, *Fusarium*, and *Penicillium*, poses significant risks to human and animal health (1). In Poland, mycotoxins such as deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), and T-2/HT-2 toxins are most frequently detected, often co-occurring in cereal grains. Ongoing climate change may further alter contamination patterns, potentially increasing the prevalence of other mycotoxins, including aflatoxins (2,3).

The aim of this study was to compare reference chromatographic methods: high-statement liquid chromatography with fluorescence detection (HPLC-FLD) or tandem mass spectrometry (HPLC-MS/MS) with two commercially available rapid strip tests for mycotoxin analysis. A total of 90 randomly selected grain samples collected in 2025 (barley n = 10), wheat (n = 21), triticale (n = 10), maize (n = 49) were analyzed for DON, ZEN, OTA, and the sum of T-2 and HT-2 toxins.

Although none of the analysed samples exceeded the maximum levels established by EU regulations, widespread contamination with one or more mycotoxins was observed. Chromatographic methods demonstrated superior sensitivity, specificity and precision, confirming their status as the gold standard for mycotoxin determination. In contrast, rapid strip tests offered advantages such as low cost, short analysis time, and ease of use, making them suitable for on-site screening and preliminary risk assessment. However, their significantly higher limits of detection and quantification limit their applicability in advanced laboratory analyses or experimental studies. Therefore, results obtained using rapid tests should be confirmed using validated chromatographic methods.

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3.9 P9 - Targeting Tenuazonic Acid: Development of a Multiplex Suspension Array Fluorescence Immunoassay for *Alternaria* and Major Regulated Mycotoxins

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Alternaria toxins are emerging mycotoxins, frequently detected in cereals, fruits and vegetables. Among them, tenuazonic acid (TeA) is the most prevalent and often quantitatively dominant compound, substantially contributing to dietary exposure. (1) Due to its toxicological relevance and frequent occurrence in tomato-based products, TeA, together with alternariol and alternariol monomethyl ether (AOH, AME) (2), has become a focus of intensified EU monitoring activities. (3) However, rapid multiplex screening tools for TeA suitable for routine food industry application remain limited. The suspension array fluorescence immunoassay (SAFIA) is a bead-based multiplex platform enabling simultaneous detection of multiple analytes within a single measurement and is particularly suited for comprehensive on-site screening of co-occurring mycotoxins. (4)

Within this work, we report the development of a novel monoclonal antibody specifically targeting TeA and its integration into a multiplex SAFIA format. A rationally designed hapten containing an elongated six-carbon linker was synthesized and coupled to TeA via oxime ligation to optimize epitope presentation. Immunization of BALB/c mice resulted in strong hapten-specific immune responses.

Monoclonal antibodies were generated using hybridoma technology. Hybridoma screening was performed using a multiplex SAFIA approach, enabling simultaneous evaluation of binding performance and antibody productivity within a single measurement. Two hapten variants with different linker lengths were applied to identify antibodies that potentially recognize the spacer, while a parallel bead-based sandwich assay allowed IgG quantification for productivity assessment. This integrated screening strategy provides functional affinity information beyond classical ELISA-based formats. More than 20 monoclonal antibodies were obtained, exhibiting EC₅₀ values between 2.0 µg/L and 100 µg/L, demonstrating a broad affinity spectrum. The most sensitive candidates indicate the feasibility of establishing detection limits in the low ppb range. Selected clones showed compatibility with ethanol-based extraction procedures, and a first proof of concept for TeA detection in tomato ketchup was successfully demonstrated.

In parallel, monoclonal antibodies against alternariol were generated and are currently under detailed characterization. Current work aims at assay optimization, matrix validation and full integration of TeA and AOH into an established multiplex SAFIA panel comprising key regulated mycotoxins such as DON, ZEN, FUM, T-2/HT-2, OTA and aflatoxins. This strategy paves the way for comprehensive, rapid multi-toxin screening that combines emerging and regulated contaminants within a single analytical platform.

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3.10 P10 - Tetramic acid derivatives from *Alternaria* and *Fusarium* counteract zearalenone-induced estrogenic signaling in 2D Ishikawa and 3D MCF-7 cells

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Mycotoxins produced by *Alternaria* and *Fusarium* species may pose potential threats to human health due to their frequent occurrence in food and their diverse biological activities. Among these, the *Alternaria* mycotoxin altersetin (AST) and the *Fusarium* mycotoxins equisetin (EQUI) and trichosetin (TRI) represent a group of decalin-containing tetramic acid derivatives, whereas tenuazonic acid (TeA; *Alternaria* mycotoxin) is a structurally related tetramic acid derivative lacking the decalin moiety. Given their shared tetramic-acid scaffold and the known antiestrogenic effects of AST [1], we investigated whether this structural motif could underlie antiestrogenic activity across related compounds, particularly under co-exposure conditions with the estrogenic *Fusarium* mycotoxin zearalenone (ZEN), which may co-occur in food.

Antiestrogenic activity of TeA, EQUI, and TRI was first assessed in 2D Ishikawa cultures using the alkaline phosphatase (ALP) assay, with cell viability monitored in parallel via CellTiter-Blue. To further validate the findings under more physiologically relevant conditions, combinatory effects of EQUI and TRI with ZEN were evaluated in MCF-7 3D spheroids by quantifying spheroid growth over 10 days. The potential formation of necrotic cores was examined by fluorescence microscopy using viability-based LIVE/DEAD[®] staining.

In 2D cultures, TeA displayed clear antiestrogenic effects in combination with ZEN starting at 2 μM without affecting cell viability. Both EQUI and TRI showed robust antiestrogenic effects in combination with ZEN, with TRI active at $\geq 1 \mu\text{M}$ and EQUI markedly more potent ($\geq 0.1 \mu\text{M}$). In 3D MCF-7 spheroids, ZEN strongly promoted spheroid expansion, whereas co-treatment with EQUI or TRI attenuated this response. EQUI reduced ZEN-induced spheroid growth by approximately 50% at concentrations $> 2 \mu\text{M}$, while TRI required $\geq 20 \mu\text{M}$ to achieve significant suppression. No necrotic core formation was observed under any treatment condition.

These findings identify TeA, EQUI, and TRI as previously unrecognized antiestrogenic mycotoxins, with EQUI emerging as the most potent. The results raise the possibility that co-exposure to ZEN and tetramic acid derivatives may attenuate ZEN's estrogenic effects under real-life exposure scenarios, a hypothesis that warrants further investigation. However, given the current lack of occurrence data for EQUI and TRI, their presence and levels in food and feed need to be clarified. Taken together, our results point toward the tetramic-acid scaffold as a potential determinant of antiestrogenic activity.

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3.11 P11 - Target fishing in the "kinome": integrated in silico/in vitro discovery of novel kinase inhibitory activities of alternariol

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The *Alternaria* mycotoxin alternariol (AOH) may contaminate food, raising concerns for food safety. Although AOH exhibits cytotoxic, genotoxic, mutagenic, and immunosuppressive effects [1], mechanistic knowledge gaps still limit its toxicological understanding, with possible consequences for risk assessment. AOH has been described as a kinase inhibitor [2] and, given the critical role of kinases in cellular signaling and toxicity mechanisms [3], understanding its kinase inhibitory profile is essential for establishing a mechanistic framework supporting its toxicological understanding.

This study presents an integrated in silico/in vitro approach to discover novel AOH target kinases. A ligand-based virtual screening was performed on a library of 3,555 kinase ligands to identify compounds structurally similar to AOH, based on the principle that similar compounds may bind to the same protein targets. This enabled us to prioritize ten kinases that were subjected to 3D molecular modeling (integrating docking, molecular dynamics simulations, and energy free binding estimates) to assess AOH-protein binding stability. Among the candidates, a short list of strong candidates possibly inhibited by AOH was outlined and included newly identified targets like PIK3CB, JAK2, and PIM1. Experimental follow-ups on Caco-2 cells confirmed computational predictions, demonstrating that AOH significantly inhibits the PI3K/AKT pathway, as monitored by AKT phosphorylation as a reporter system. Additional computational analysis revealed that AOH glucuronide metabolites exhibited reduced binding stability compared to the parent compound, suggesting phase II metabolism may modulate AOH kinase inhibitory activity.

These findings provide important mechanistic insights into AOH toxicology and demonstrate the value of integrated in silico/in vitro approaches to improve the toxicological understanding of mycotoxins.

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3.12 P12 - Interinstitutional Network for Biomonitoring Citrinin Exposure in Children and Adolescents: Fostering Interdisciplinary Collaboration Between Portugal and Germany

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Citrinin (CIT), a nephrotoxic mycotoxin produced by *Aspergillus*, *Penicillium*, and *Monascus* species, frequently contaminates cereals and represents a potential health concern for children and adolescents, who consume relatively higher amounts of cereal-based products per body weight.[1] The CITChild project establishes a bilateral interinstitutional collaboration between research teams in Portugal (REQUIMTE) and Germany (University of Münster) to comprehensively assess CIT exposure through biomonitoring in urine and analysis of processed foods. Besides CIT, its metabolite dihydro-citrinone (DH-CIT), and the thermal degradation product decarboxy-citrinone (DCIT), including bound forms in cereals are investigated.

This initiative integrates complementary expertise in analytical chemistry, toxicology, nutrition, risk assessment, and public health. Joint efforts encompass the development and standardization of sensitive analytical methods for urine and food, cross-lab validation with exchanged standards and quality controls, mutual research stays for method harmonization and training. Building on this collaborative framework, exposure data will be generated from urine samples and cereal-based food items per country, combined with dietary habit surveys. The interdisciplinary approach yields comprehensive datasets on exposure levels, dietary influences, country-specific differences, and potential risks, supporting EFSA-aligned assessments and evidence-based food safety policies. Key outcomes include advanced biomonitoring tools, educational resources on mycotoxins, and a sustainable Portugal-Germany research network promoting long-term cooperation, researcher mobility, and capacity building.

Here we will present data on method harmonization and cross validation and first results of cereal contamination assessment.

3.13 P13 - Atranone – an overlooked secondary metabolite?

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Atranones are secondary metabolites of the mold *Stachybotrys* spp., which are particularly prevalent in water-damaged indoor environments. Atranones have received little attention compared to the more toxic macrocyclic trichothecenes. Approximately 60% of all *Stachybotrys chartarum* strains isolated from indoor environments produce atranones, while only 40% form macrocyclic trichothecenes (1).

Since simultaneous synthesis of macrocyclic trichothecenes and atranones has not yet been observed, the production of these mycotoxins seems to be mutually exclusive. Atranones are dolabellane-like diterpenoids with a complex chemical structure. Starting from geranylgeranyl pyrophosphate, the biosynthesis of atranones requires several enzymatic steps and the corresponding enzymes are encoded in the atranone-specific core gene cluster. Although the structure of this gene cluster is known, the regulation of its genes is still poorly understood.

Experimental studies in cell culture and animal models show that atranones possess pronounced pro-inflammatory and cytotoxic effects, particularly in the respiratory system. In murine models, they seem to induce inflammatory cell infiltration and the expression of pro-inflammatory cytokines, while necrotic cell death, apoptosis, and cell cycle arrest have been observed in human cell lines. For atranone Q, a pronounced antitumor effect against osteosarcoma cells was reported based on *in vitro* studies. More recently identified derivatives, such as stachatranone and stachybatranone, seem to have cardioprotective effects under ischemic conditions. Despite the proven pro-inflammatory and cytotoxic properties, as well as the pharmacological potential of atranones, their scientific investigation remains insufficient compared to macrocyclic trichothecenes. A key open question is whether atranones are actually formed under real-world indoor conditions, as they have not yet been detected there. Epidemiological data, dose-response relationships, and exposure limits are lacking to assess their potential health effects.

Due to their biological, chemical, and pharmacological properties, atranones may possess a currently underestimated medical potential, making more intensive research on these mycotoxins urgently necessary (2).

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3.14 P14 - Multi-country survey of major mycotoxins in maize, sorghum and millet across Africa within the UP-RISE framework

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Mycotoxins are toxic secondary metabolites produced mainly by *Aspergillus*, *Fusarium* and *Penicillium* species, contaminating food and feed worldwide and posing a major threat to food safety and security. Among them, aflatoxins, fumonisins, trichothecenes, ochratoxin A and zearalenone are considered the most harmful. Hot and humid climates, grain damage during harvest, and inadequate post-harvest handling and storage contribute significantly to fungal proliferation and toxin accumulation, particularly in cereals.

In Africa, aflatoxins (AFLA) and fumonisins (FBs) are the most widespread contaminants in staple cereals such as maize, sorghum and millet. However, limited surveys have also reported the occurrence of other regulated mycotoxins, including deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEN). Mycotoxin concentrations in several African cereal value chains frequently exceed the maximum limits set by the European Union, while national regulatory frameworks and routine monitoring systems remain insufficiently developed. These gaps pose substantial challenges for ensuring safe raw materials and high-quality products for both human and animal consumption. Strengthening surveillance and integrating mycotoxin management into agricultural and food system policies is therefore essential.

Within the UPRISE project, this study presents a comprehensive survey of major mycotoxins—AFLA, FBs, trichothecenes, ochratoxin AFLA, FBs, DON, OTA and ZEN in maize, sorghum and millet collected during harvest and storage in five African countries (Benin, Côte d'Ivoire, Nigeria, Kenya and South Africa), representing diverse agro-ecological zones. Rapid on-site screening was performed using commercial lateral flow assay (LFA) kits to enable high-throughput testing close to production and storage locations. In this study, we present the first crop-season results based on a total of 607 samples collected at harvest and 276 samples during storage. Maize emerged as the most contaminated matrix, with AFLA and FBs being the most prevalent mycotoxins across the surveyed countries, while a sporadic detection of DON, OTA and ZEN was observed. However, a co-occurrence of the five tested mycotoxins was observed in 5% of sorghum and millet samples collected during harvesting in Kenya.

The obtained data provide an updated overview of contamination patterns and geographical variability, highlighting country- and crop-specific risk profiles. These findings, combined with those of the second crop season still ongoing, contribute to the development of targeted mitigation strategies and support the adoption of scalable monitoring approaches for enhanced food safety in African cereal value chains. Moreover, the analysis of the collected data will help identify persistent challenges and gaps in mycotoxin management, offering valuable recommendations to strengthen monitoring capacities, inform policy decisions and improve food safety governance across the African region.

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3.15 P15 - Exploring mycotoxin interactions with heme transporters in Atlantic salmon: a computational journey

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Over the last decade, Atlantic salmon aquafeeds have increasingly incorporated plant-derived ingredients, especially cereals, impacting the chemical hazard landscape and increasing exposure to crop-associated mycotoxins [1]. Among these, enniatin B (ENNB) and beauvericin (BEA) are repeatedly detected in feed matrices and have been associated with health-relevant outcomes in Atlantic salmon, including impaired growth and anemia-like phenotypes [2]. However, the molecular events linking dietary exposure to these outcomes remain insufficiently resolved, limiting mechanism-driven hazard interpretation and prioritization.

Beyond heme biosynthesis, cellular heme availability is controlled by heme turnover, which in turn is (partially) controlled by proteins acting as heme transporters. This impacts heme distribution, detoxification, and iron handling, thus possibly influencing the anemia-like outcomes [3].

Here, we apply a computational multi-target strategy to explore whether ENNB and BEA can plausibly interact with representative salmon proteins involved in heme transport, including FLVCR1, FLVCR2, HMOX1, and SLC46A1. Using Boltz-2, an AI-based protein 3D modelling software, followed by molecular dynamics simulations, we evaluated binding plausibility and interaction stability over time. The analysis showed potentially stable interactions for both compounds across the *in silico* tested targets, obtaining plausible groundwork for further experimental validation. Overall, this work supports a mechanism-driven interpretation of emerging mycotoxin risks in modern salmon feeds and provides a testable roadmap to guide targeted follow-up studies in salmon-relevant systems. This study falls within the MYTOXA project (Norwegian Research Council, grant number 34401), integrating *in vivo*, *in vitro*, and *in silico* approaches to assess how feedborne exposure to emerging mycotoxins influences salmon growth and smoltification during the freshwater phase.

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3.16 P16 - *In Vitro* Binding Evaluation of Silicoglycidol, a Mycotoxin Binding Technology Under Simulated Gastrointestinal Conditions of Broiler Chickens

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Mycotoxin contamination of poultry feed poses a continuous risk to animal health, productivity, and food safety. This study evaluated the *in vitro* binding efficacy of a Silicoglycidol-based mycotoxin binder against multiple mycotoxins under simulated broiler gastrointestinal conditions. Contaminated maize-based feed was supplemented with Silicoglycidol at 0.5 kg/ton and subjected to a multi-step *in vitro* digestion model representing gastric and intestinal phases. Binding efficacy was qualitatively assessed by Fourier-transform infrared spectroscopy (FTIR) and quantitatively confirmed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). All experiments were conducted in triplicate, and phase-dependent differences were analyzed using paired t-tests. FTIR analysis demonstrated strong adsorption of deoxynivalenol (DON), aflatoxins, and T-2 toxin, as indicated by the disappearance or attenuation of characteristic spectral peaks. LC-MS/MS results showed near-complete binding of aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂; 99–100%) in both gastric and intestinal phases, with no statistically significant differences between phases ($p > 0.05$). Similarly, DON (94–99%) and ochratoxin A (88–95%) exhibited consistently high binding efficiencies across gastrointestinal conditions, with no significant phase-dependent variation ($p > 0.05$). Zearalenone, fumonisin B₁, fumonisin B₂, and T-2 toxin maintained high binding efficiencies under both gastric and intestinal conditions, demonstrating stable adsorption performance across simulated broiler gastrointestinal phases. Overall, Silicoglycidol demonstrated robust and broad-spectrum mycotoxin-binding capacity under simulated broiler gastrointestinal conditions. The observed statistically significant differences reflect expected physiological phase-related variation rather than reduced functional efficacy. These findings support the suitability of Silicoglycidol as an effective mycotoxin mitigation strategy in poultry and animal nutrition.

Keywords: Mycotoxin binder; Silicoglycidol; Broiler gastrointestinal simulation; Feed safety; LC-MS/MS analysis

3.17 P17 - Simulating the Upper and Lower Gastrointestinal Tract of a Cereal-Based Sample: An In Vitro Study on modified HT-2 and T-2 Toxins

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Currently, there is insufficient data on the bioavailability of modified HT-2 and T-2 mycotoxins, as only the monoglucosides have been studied to date [1, 2]. The aim of this study was therefore to simulate the upper and lower gastrointestinal tract (GIT) on a cereal-based sample to obtain more detailed information on the release of HT-2 and T-2 toxins bound to polysaccharides present in cereals. The concentration of free forms of HT-2 and T-2 toxins was first determined in the samples. The same cereal sample was then subjected to 44 hours laboratory enzyme hydrolysis, which had been previously optimized at the research site and can release the majority of modified forms. This laboratory hydrolysis was used to compare the release of free forms of mycotoxins with simulated GIT. To simulate the upper GIT, hydrolysis was performed according to the method described by Gratz et al. [3] using artificial digestive juices – artificial saliva, gastric juices, NaHCO₃, and duodenal juices. Part of the oat pellets produced after simulation of the upper GIT was subsequently used for simulation of the lower GIT. For that, colon microbiota isolated from patient stool samples was used in collaboration with Motol University Hospital. The lower GIT simulation procedure based on a publication by Dell'Olio et al. [4] was optimized, in which a basal culture medium was created and added to the pellet together with human faecal microbiota. The mixture was incubated (24 h, 37 °C) in an anaerobic environment in an anaerostat. The samples were then analysed using U-HPLC-HRMS/MS and to account for potential matrix effects, isotope-labelled standards were used. The results will be presented in detail at the conference.

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3.18 P18 - Volatile apocarotenoids treatment in management of *Fusarium oxysporum* f. sp. *lini* infection

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Flax cultivation is limited by susceptibility to *Fusarium oxysporum* f. sp. *lini*, which causes Fusarium wilt (1). β -cyclocitral (β CC) is volatile product of β -carotene oxidation in plants (2). It significantly enhances plant's tolerance to both abiotic (3) and biotic stresses (4). However its role in plant's response to fungal infection remains unknown. We investigated the efficacy of treatment with β CC and its derivatives (β -cyclocitric acid and 2,2,6-trimethylcyclohexanone) in managing infection.

All three compounds exhibited concentration-dependent fungicidal activity in vitro, significantly reducing mycelial growth and biomass. In planta, optimized treatment suppressed early infection stages and lowered overall disease severity. Treatment also inhibited the characteristic red pigmentation of fungal mycelium, indicating reduced production of bikaverin. It is a secondary metabolite classified as a mycotoxin and produced by *Fusarium* species (5). The decrease in bikaverin suggests that these compounds directly modulate fungal secondary metabolism and may also influence the biosynthesis of other mycotoxins.

In conclusion, these findings identify β -cyclocitral and its derivatives as promising antifungal agents with potential application in sustainable management of Fusarium wilt in flax.

Keywords: β -cyclocitral, apocarotenoids, *Linum usitatissimum* L., *Fusarium oxysporum* f.sp. *lini*, Fusarium wilt

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3.19 P19 - Degradation of mycotoxin AFB₁ using bacterial isolate *Rhodococcus* sp. (SFFA/2)

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Diverse environmental conditions lead to the production of fungal secondary metabolites- mycotoxins in food and feed materials. Among these most common are aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), deoxynivalenol (DON), fumonisins (FB₁, FB₂, FB₃), HT-2 toxin, ochratoxin A (OTA), T-2 toxin, and zearalenone (ZEN). AFB₁ is regulated globally and is considered among the most economically important mycotoxins in terms of their high prevalence and significant negative effects on animal performance. AFB₁ is mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* on corn, wheat, oats and other crops of agricultural importance. Remediation of AFB₁ in animals can be achieved by using adsorbents or biotransformation agents (microbes/enzymes) to reduce the carryover to humans. In this study, an AFB₁ degrading microbe was isolated from an environmental sample utilizing enrichment culture procedure, using Inorganic Salt Culture medium (ISC), and a mix of mycotoxins (AFB₁, FB₁, DON, ZEN, OTA, T-2/HT-2), 1 ppm each, as a sole carbon source. After the first round of enrichment, the level of AFB₁ mycotoxin was reduced. In the next round, only 1 ppm of AFB₁ was added. Procedure was repeated multiple times and samples were taken after 24 and 48 hours for analysis of AFB₁ using LC-MS/MS. Isolated bacterial culture SFFA/2 (Lab code), was identified by NCIMB (UK) belonging to the *Rhodococcus* genus. The most suitable temperature for degradation of AFB₁ is 30°C and optimal pH is between 5–7. *Rhodococcus* sp. degrades 60.4% and 98.2% of 1 ppm of AFB₁ toxin in 24 and 48 h respectively in ISC. This microbe will degrade 19.1% of 1 ppm of AFB₁ stock extracted from lab contaminated material after 48 h. SFFA/2 degrades 9.3% of 0.3 ppm of AFB₁ from lab contaminated material, at 30°C, after 24 h, and 16.7% in 48 h. Degradation results for stability showed that *Rhodococcus* sp. is resistant to a 3-minute treatment with high temperatures (80-90°C) when dried. The metabolite produced because of biotransformation of AFB₁, could not be identified. 10⁸ CFU/ml of viable bacteria will degrade 98.6% of AFB₁ in 48 h, and 10⁷ CFU/ml results in 49.3% reduction in 48 h. Based on this data bacterial culture SFFA/2, identified as *Rhodococcus* sp. has a potential for use in animal feed to reduce the absorption of AFB₁ in animals and their carryover from animals to humans.

Keywords: AFB₁, LC-MS/MS, mycotoxins, degradation, *Rhodococcus* sp.

3.20 P20 - LC-MS/MS analysis of aflatoxin B₁ and fumonisin B₁ in maize and black soldier fly larvae

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Climatic stress factors such as high temperature, drought and inadequate storage conditions pose a major challenge for food and feed safety in sub-Saharan countries, particularly leading to contamination of maize with mycotoxins^{1, 2}. At the same time, the increasing demand for alternative food and feed sources has stimulated research into the use of insects as sustainable protein sources as well as for their potential to influence mycotoxin fate in contaminated substrates^{3, 4}. However, data on the transfer of mycotoxins from feed to insect larvae and their toxicological implications remain limited^{5, 6}. Therefore, reliable analytical methods are essential to quantify mycotoxin concentrations in insect matrices and assess potential carry-over.

An LC-MS/MS method for the determination of aflatoxin B₁ (AFB₁) and fumonisin B₁ (FB₁) in maize and larvae of the black soldier fly (*Hermetia illucens*, BSF) was developed and validated. AFB₁ and FB₁ were extracted from maize using a simplified QUEChERS approach with minimal sample preparation. The BSF larvae were extracted using a simple solvent-based extraction with acetonitrile for AFB₁ and methanol for FB₁. Chromatographic separation was performed using LC-MS/MS in multiple reaction monitoring with positive electrospray ionization.

Validation results showed recovery rates for both mycotoxins with values of approximately 80-90% in spiked maize and 65-80% in spiked BSF larvae. Precision and repeatability were within accepted limits, with relative standard deviations below 10% for maize and below 5% for larvae. The results highlight the need for matrix-specific extraction strategies, as the insect matrix, due to its complex composition, required more intensive sample preparation (cryogenic grinding) for reliable quantification. The developed method provides detection limits for both analytes that are sufficiently low for application in food and feed safety studies. The developed method will be applied on ongoing collaborative research on the fate of mycotoxins along processing chains under local conditions in Kenya.

Within a German-Kenyan cooperation project, various samples from an insect-based production system in Kenya will be analyzed to evaluate the mycotoxin transfer rates under local conditions. The data will contribute to a better understanding of risks along the value chain from contaminated maize over a potentially detoxifying step in edible insects further on to the use of the larvae in fish feed production and support the development of guidelines for safe use of insect proteins in regions highly affected by mycotoxin contamination.

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3.21 P21 - Mycotoxins and plant toxins in plant protein concentrates used for meat alternatives in the German market

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Plant-based meat alternatives have seen an increase in consumer popularity in the recent years. This led to the industrial use of concentrated protein sources such as wheat gluten, soy isolates and pea isolates. Because these ingredients are highly processed isolates, the natural toxins such as mycotoxins and plant toxins found in the original grains or beans can be concentrated into much higher levels in the final products. The presence/co-occurrence of these mycotoxins and plant toxins in these plant-based meat alternatives can pose health risks to consumers [1-3].

Currently, aflatoxins and ochratoxin A are regulated by the European Union (EU) in processed cereal products. In addition, ergot alkaloids are regulated by the EU in wheat gluten. However, the presence of other toxins e.g. fumonisins and *Alternaria* toxins has also been reported [2].

A LC-MS/MS method for the simultaneous determination of a wide range of mycotoxins and plant toxins (114 toxins) in wheat gluten and further protein isolates has been developed and validated according to the latest EU regulations for mycotoxins (EU 2023/2782) and plant toxins (EU 2023/2783). The method involved QuEChERS-based extraction and LC-MS/MS analysis using triple quadrupole mass spectrometry.

The toxins covered by the method include aflatoxins, trichothecenes (nivalenol and related compounds, T-2 toxin and related compounds, among others), fumonisins, zearalenone and related compounds, beauvericin, enniatins, ochratoxins, *Alternaria* toxins, ergot alkaloids as well as tropane alkaloids, pyrrolizidine alkaloids, and quinolizidine alkaloids.

The validated LC-MS/MS multi-method proved to be a reliable and efficient tool for the simultaneous determination of multiple mycotoxins and plant toxins in plant proteins. It was applied to samples collected from the German market. For wheat gluten, the results revealed the presence of *Alternaria* toxins, deoxynivalenol, enniatins, ergot alkaloids and ochratoxin A.

The aim of this work was to investigate the levels of several mycotoxins and plant toxins and their co-occurrence in plant proteins used for meat alternatives. In contrast to other studies, the focus was on the presence of toxins in the protein isolates themselves in order to avoid confounding factors, i.e. the presence of toxins from other ingredients (e.g. fats, spices) of meat alternatives.

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3.22 P22 - Addressing Multi-Mycotoxin Analysis in Complex Food Matrices: Validation of a Multiplex Flow Cytometric Immunoassay

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The co-occurrence of multiple mycotoxins in food and feed poses a persistent food safety challenge, as their toxic effects can be additive or synergistic and regulatory limits span diverse commodities. This complexity creates a pressing need for multiplex analytical methods capable of simultaneously quantifying several mycotoxins with sensitivity and robustness across varied matrices (1, 2). In this study, a multiplex suspension array fluorescence immunoassay with flow-cytometric detection was systematically evaluated for the simultaneous quantification of six regulated mycotoxin groups: aflatoxins (B1, B2, G1, G2), ochratoxin A (OTA), deoxynivalenol (DON), fumonisins (FB1–FB3), zearalenone (ZEN), and the sum of T-2/HT-2 toxins.

Method performance was assessed following the Eurachem *Fitness for Purpose of Analytical Methods* framework (3), with particular emphasis on calibration behaviour, detection capability, quantitative working range, trueness, precision, measurement uncertainty, and method ruggedness. The validation deliberately covered a broad spectrum of food matrices, including high-protein, high-fat, high-sugar, high-water, and processed products, reflecting realistic routine testing conditions. Quantification relied on sigmoidal four-parameter calibration models with automated data evaluation to account for the non-linear response characteristics of competitive immunoassays.

Based on extensive blank measurements, the limits of detection (LOD) were 0.883 µg/kg for aflatoxins (AFL), 1.16 µg/kg for OTA, 3.49 µg/kg for ZEN, 11.3 µg/kg for T-2/HT-2 toxins, 39.4 µg/kg for FUM, and 47 µg/kg for DON. Experimentally confirmed working ranges spanned 0.9 – 15 µg/kg (AFL), 1.2 – 50 µg/kg (OTA), 3.5 – 300 µg/kg (ZEN), 11.5 – 300 µg/kg (T-2/HT-2), 50 – 700 µg/kg (FUM), and 50 – 1500 µg/kg (DON). Across all analytes and matrices, recoveries met regulatory fitness criteria (Mean values spanning from 94% (FUM) to 100% (ZEN)), while intra-assay and intermediate precision remained below 10% and 20% RSD, respectively. Expanded measurement uncertainty (MU95, $k = 2$) ranged from 35% (ZEN) to 51% (T-2/HT-2).

These results demonstrate that multiplex immunoassay-based approaches can deliver robust, quantitative multi-mycotoxin data under routine analytical conditions, supporting efficient food safety monitoring in complex matrices.

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3.23 P23 - High-Throughput Profiling of Mycotoxigenic Fungi in Spanish Cereal Soils

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Although mycotoxin-producing fungi have been extensively studied in food commodities, soil remains comparatively underevaluated, despite being a critical reservoir within the food production chain. Characterizing the soil mycobiome is key to mitigating contamination risks. In this work, high-throughput amplicon sequencing was used to analyze the mycobiota of Spanish cereal field soils using internal transcribed sequence 2 (ITS2) to assess fungal diversity and elongation factor 1 alpha gene (TEF1) to specifically evaluate *Fusarium* species and compare taxonomic resolution. Effects of cereal crop (wheat, oat, barley), soil pH and location were assessed. Sequence data were processed with DADA2 (1) and analyzed in R using phyloseq (2).

Based on ITS2 amplicon sequencing, *Fusarium* was the most abundant genus (18.42%), followed by *Cladosporium* (15.56%), *Penicillium* (12.03%), and *Alternaria* (6.59%), with all these genera except *Cladosporium* known as mycotoxin producers. Alpha diversity differed significantly between wheat and barley soils and between basic and neutral soils. Community composition, assessed using Aitchison distances (3), was mainly structured by location and soil pH. Differential abundance analysis revealed pH-associated patterns among mycotoxin-producing genera. *Aspergillus* genus was enriched in alkaline soils, whereas *Penicillium* genus predominated in acidic conditions.

Due to the limited species-level resolution of ITS2 within *Fusarium*, particularly in closely related complexes, TEF1 was used to improve taxonomic discrimination. TEF1 identified 37 *Fusarium* species compared to 18 with ITS2, with only 8 shared taxa with differences in relative abundance. Two members of the *Fusarium incarnatum-equiseti* species complex (FIESC) dominated depending on the marker: *F. brevicaudatum* in the ITS2 analysis and *F. clavum* in the TEF1 case. The latter species has been previously reported in Spanish cereal fields (4). The *Fusarium sambucinum* species complex (FSAMSC) represented the largest proportion of species detected, and TEF1 showed higher resolution within this complex (10 species vs. 4 with ITS2). Other important *Fusarium* taxa detected with TEF1 included *F. oxysporum*, *F. proliferatum* and *F. langsethiae*, all known mycotoxin producers. Species composition was influenced by soil pH. *Fusarium clavum* and *F. equiseti* were enriched in alkaline and neutral soils, respectively, whereas *F. oxysporum* was associated with acidic conditions. Among cereals, only *F. clavum* was significantly more abundant in wheat than in oat soils. Overall, these findings highlight soil as a key reservoir of mycotoxigenic species and support TEF1 as a more suitable marker for species-level discrimination within *Fusarium* genera.

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3.24 P24 - Analysis of 10 mycotoxins in different agricultural and food/feed matrices using LC-MS/MS

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In this article a multi-mycotoxin analysis method based on solid-liquid extraction (SLE) for analysis of aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), deoxynivalenol (DON), fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), ochratoxin A (OTA), T-2 toxin, and zearalenone (ZEN) in various agricultural (corn, wheat, barley, wheat bran, soya bean, sunflower seed) and food/feed matrices (compound feed for pigs and poultry and total mixed ration for dairy cows) was developed and validated using LC-MS/MS Triple Quadrupole (Waters Xevo TQ-XS) at Betagro Science Center, Thailand. The samples were finely grounded and homogenized using a laboratory mill and 2.5 g sample was weighed and placed in a conical tube. To this tube, 10 ml extraction mixture I (80% acetonitrile:19.9% water:0.1% formic acid) was added and the lid was closed. This tube was placed on an orbital shaker at 230 rotations per minute (rpm) for thorough mixing (90 mins at room temperature). The tube was then centrifuged at 4000 × g for 5 mins at 4°C and the supernatant was removed to another 50 ml tube. The 500 µl of supernatant was pipetted and combined with 500 µl of diluent solvent (50% acetonitrile:0.1% formic acid) in 15 ml tube and mixed solution by vortexing for 30 s. To compensate for the matrix effects during electrospray ionization, the mixed ¹³C labelled internal standards for aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), deoxynivalenol (DON), fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), ochratoxin A (OTA), T-2 toxin, and zearalenone (ZEN) were pipetted (20 µl) into 1000 µl of the extract contained in 15 ml tube and mixed solution by vortexing for 30s. A 1020 µl liquid was prepared by filtering the extract through a membrane syringe filter into a glass vial for LC-MS/MS analysis. The recoveries of 10 mycotoxins spiked in food/feed and cereal matrices were recorded in the range of 93.6%–114.2% after internal standard correction (relative standard deviations (RSDs) below 14.2%). This data proves that this is a robust method based on **dilute and shoot** technique for simultaneous analysis of 10 mycotoxins using LC-MS/MS.

Key words: LC-MS/MS, multi-mycotoxin, food, feed.

3.25 P25 - Metataxonomic profiling of *Tenebrio molitor* gut microbiota after exposure to aflatoxin B1, fumonisin B1, and deoxynivalenol

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Mycotoxin contamination represents a major challenge for global food security and waste management (1)(2). Sustainable strategies for agricultural waste management are essential for the transition toward a circular economy, and the frequent contamination of these by-products with mycotoxins poses a significant challenge for their safe valorisation. *Tenebrio molitor*, known as yellow mealworm, has gained importance not only as a sustainable protein source for human food and animal feed but also as a promising agent for the bioconversion of organic waste (3). Whereas the capacity of *T. molitor* larvae to tolerate and develop on contaminated substrates is documented, the impact of dietary mycotoxins on its gut microbial community remains to be fully characterized. This study investigated the effects of aflatoxin B1 (AFB1), fumonisin B1 (FB1), and deoxynivalenol (DON) supplemented diets on the composition of the gut microbiota of *T. molitor* larvae. Larvae were reared on contaminated wheat bran and carrots for 44 days. To evaluate culture-independent microbial community dynamics, total genomic DNA was extracted from the intestinal tracts, followed by a 16S rRNA gene metataxonomic analysis. Although no significant differences were observed in the overall diversity of the microbial community exposed to mycotoxins, changes occurred in specific groups. The differential abundance analysis revealed specific bacterial groups that are overrepresented in the gut of *T. molitor* larvae reared on mycotoxin-supplemented substrates. Therefore, these groups which became more prevalent following exposure to mycotoxins might be potentially involved in detoxification. In particular, *Stenotrophomonas* spp. levels significantly increased in the FB1-exposed group, whereas *Enterococcus* spp. and *Mixta* spp. were enriched in the microbiota of larvae exposed to AFB1. Conversely, the presence of *Klebsiella* sp. showed a significant reduction in the DON- and AFB1-exposed groups, while *Lactococcus* was significantly reduced in the FB1 group. These findings support the potential of the gut microbiota of *T. molitor* as a reservoir of candidate microorganisms for future targeted isolation of possible decontaminating agents.

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3.26 P26 - Molecular Determinants of Functional Tenuazonic Acid Immunoassays: Sequence–Structure Correlates of Competitive Performance

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Tenuazonic acid (TeA) is one of the most prevalent *Alternaria* toxins in food and feed, frequently occurring in cereals, tomato products, and fruit-based commodities (1). Due to its widespread occurrence and potential toxicological relevance, reliable analytical methods for its detection are essential. Immunochemical assays like suspension array fluorescence immunoassay (SAFIA) or competitive indirect lateral flow immunoassays (LFIA) offer rapid and cost-effective screening. However, variability in antibody performance can significantly affect assay sensitivity, selectivity, and robustness. The molecular determinants governing functional suitability of antibodies in competitive TeA assays remain largely unresolved (2).

To elucidate sequence–structure features underlying assay performance, 22 monoclonal antibodies were analyzed by integrating competitive ELISA data, affinity measurements by microscale thermophoresis, and comprehensive VH/VL sequence analysis. Structural models of the variable domains were generated using AlphaFold and subjected to docking simulations with TeA via HADDOCK 2.4. To assess analytical selectivity and potential interference risks, cross-reactivity was investigated against structurally related compounds.

Sequence clustering revealed a strict correlation between competitive ELISA functionality and a defined VH/VL-CDR3 motif combination. All antibodies suitable for competitive assay formats carried the heavy-chain CDR3 motif **AREGGY** together with the light-chain CDR3 motifs **AQSIHFPYT** or **AQSSHVPYT**. Single-point mutation of the heavy-chain motif (E→F; ARFGGY) or elongation of CDR3 regions consistently resulted in loss of functional suitability despite retained binding activity. These findings indicate that competitive TeA detection requires a highly constrained paratope geometry rather than affinity alone. Structural modelling suggests that electrostatic and aromatic interactions within this motif pair stabilize hapten accommodation, whereas minor sequence alterations disrupt this architecture and compromise assay performance.

By linking defined VH/VL-CDR3 motifs to measurable functional outcomes, this study provides mechanistic insight into performance variability of TeA immunoassays and establishes evidence-based criteria for antibody selection. The integrative workflow will be extended to additional mycotoxins, including patulin and alternariol (AOH), to enable rational optimization of immunochemical detection across diverse toxin classes.

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3.27 P27 - Rhizobacterial VOCs and their potential to counteract mycotoxigenic fungi

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Fungal diseases represent a major biotic constraint to cereal production, limiting yield and compromising the quality and safety of grains and derived products. *Fusarium* and *Aspergillus* are fungal genera particularly notable for their involvement in cereal infection and subsequent mycotoxin contamination of kernels. Furthermore, the burden of fungal diseases affecting cereals is influenced by climate change, which can alter the distribution of phytopathogenic fungal species, their interactions with host plants, and host plant susceptibility.

In this context, biocontrol strategies based on plant growth-promoting rhizobacteria (PGPRs) are emerging as a sustainable approach. PGPRs are characterized by their ability to enhance plant growth and contribute to plant defense through multiple mechanisms, including the release of volatile organic compounds (VOCs). Of particular interest are rhizobacteria originating from the rhizosphere of perennial cereals, whose ability to counteract phytopathogenic fungal species through VOCs release remains largely unexplored.

To investigate the inhibitory potential of rhizobacterial VOCs, ten bacterial strains isolated from the rhizosphere of the OK72 (*Triticum aestivum* × *Thinopyrum ponticum*) perennial wheat line were selected (1). Bacterial strains were tested against *Aspergillus flavus*, *Fusarium proliferatum*, and *Fusarium verticillioides* in a two-sealed-plate assay to allow exclusively VOC-mediated interactions.

Most of the tested bacterial strains inhibited fungal growth to varying extents depending on the fungal species tested. Semi-quantitative profiling of emitted VOCs by HS-SPME/GC-MS allowed their tentative identification based on mass spectral matching and linear retention indices. Identified compounds include alcohols, organic acids, ketones, and sulphur-containing compounds, supporting a role of VOCs in mediating the observed antifungal activity.

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3.28 P28 - Determination of ochratoxin A in pistachio and cheese: the inter-laboratory validation study

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Over the past five years, mycotoxin regulations have advanced significantly, introducing new provisions for maximum levels, sampling, and analysis (1). Among mycotoxins, ochratoxin A (OTA) has received particular attention due to its widespread occurrence in a diverse range of foods. The availability of standardized analytical methods is essential for official laboratories conducting regulatory controls (2). However, a gap remains for validated multi-matrix methods, especially for certain foods of animal origin and nuts.

This study aimed to address this gap by developing and validating a method for the determination of OTA in cheese and pistachios. OTA contamination can arise either directly from fungal infection of plant substrates or during the curing process of animal-derived foods. Notably, OTA has been detected in cured pork products and hard cheeses. The Italian National Reference Laboratory (NRL), in collaboration with Istituto Zooprofilattico Sperimentale (IZS), undertook a single-laboratory validation study. The study included assessment of the working range, evaluation of different brands of immunoaffinity columns, and verification of method performance. Homogeneity and stability checks were conducted on selected batches of contaminated pistachios and cheese, which were then characterized for use in an inter-laboratory validation study (IVS) (3).

The single-laboratory validation demonstrated satisfactory performance across the tested working range for both cheese and pistachio matrices. The evaluation of immunoaffinity columns identified suitable brands for reliable OTA extraction and quantification. Homogeneity and stability tests confirmed the suitability of the selected materials for use in proficiency testing. In May 2025, the IVS was launched, engaging 21 public laboratories from nine different EU countries.

The developed method provides a robust and validated option for OTA determination in cheese and pistachios, addressing a critical gap in official control capabilities. The successful launch and participation in the IVS highlight the method's applicability and the importance of collaborative validation efforts (4).

This work is highly relevant to food safety authorities and official control laboratories, supporting harmonized mycotoxin monitoring and enforcement across the EU. The study also underscores the need for continued development and validation of multi-matrix methods to keep pace with evolving regulatory requirements and food safety challenges.

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3.29 P29 - Mycotoxins modulate oxaliplatin-induced phagocytosis of colon cancer cells via oxidative stress

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Phagocytosis of cancer cells is pivotal during tumor development as well as in the anticancer immune response. Phagocytes can internalize and clear tumor cells and thereby stimulate an adaptive immune response by acting as antigen-presenting cells. To evade phagocytosis, tumor cells express “don’t eat me” signals or inhibit “eat me” signals. This can be overcome by drugs that induce immunogenic cell death, such as oxaliplatin, which leads to increased expression of “eat me” signals and inhibits “don’t eat me” signals. However, not only drugs can modulate the immune response, but also prevalent mycotoxins were reported to stimulate or suppress immune functions. Consequently, the question arises whether immunomodulating mycotoxins affect the ability of cancer cells to evade phagocytosis and modulate oxaliplatin-induced phagocytosis. Colon cancer cells HCT116 and SW480 cells were treated with oxaliplatin (10-100 μM), alternariol (5-20 μM), deoxynivalenol (1-4 μM), ochratoxin A (OTA) (10-40 μM), or zearalenone (ZEN) (10-40 μM) for 24h. After which phagocytosis by immature dendritic cells (DCs) generated from peripheral blood mononuclear cells was assessed by co-culture, followed by fluorescence staining and flow cytometry. Expression of “eat me” (calreticulin and phosphatidylserine) and “don’t eat me” signals (CD47 and PDL1) was investigated by flow cytometry. In addition, cell surface patterns in the form of high-mannose N-glycans were measured via DC-SIGN binding and flow cytometry. Oxidation of phospholipids was assessed using fluorescent probes and malondialdehyde content. Our results showed that OTA, ZEN (>20 μM) and oxaliplatin (>50 μM) increase phagocytosis of cancer cells, with OTA and ZEN exacerbating the effects of oxaliplatin when combined. Classical “eat me”/“don’t eat me” signals showed no clear correlation with phagocytosis-induction by OTA and ZEN. Instead, elevated phagocytosis was mainly associated with increased reactive oxygen species and upregulation of oxidized phospholipids. In conclusion, mycotoxin contamination might modulate cancer cell phagocytosis via oxidative stress induction, thereby altering the anticancer immune response and the response to immunogenic cell death inducing anticancer drugs.

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3.30 P30 - Analysis of sterigmatocystin in a controlled carry-over study in dairy cows

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Mycotoxin-contaminated feed can lead to mycotoxin contamination of animal-derived products like milk and thereby contribute to human exposure. Thus, the so called carry-over is crucial also for consumer safety assessment [1]. While carry-over of aflatoxin B₁ (AFB₁) via biotransformation to aflatoxin M₁ being present in milk and milk products is well described for ruminants [1], data on the feed-to-food transfer of other toxicologically relevant mycotoxins is limited. STC occurrence has been described in various plant-derived commodities, including cereals and cereal products, nuts and legumes, and it has also been reported in animal-derived matrices such as cheese. Sterigmatocystin (STC), a biosynthetic precursor of AFB₁, is considered genotoxic, yet information on its bioavailability, metabolism and transfer behaviour in lactating ruminants is scarce [2]. *In vitro* data from human and rat hepatic microsomes suggest that STC is predominantly metabolised via aromatic hydroxylation to catechol metabolites, like 9-hydroxy-STC, whereas furofuran epoxidation appears to be a minor pathway [3], in contrast to AFB₁. While milk is often the key matrix for carry-over considerations, blood serum can provide complementary insight into systemic availability, distribution, and potential protein binding. Moreover, a human biomonitoring study detected STC in plasma only after β -glucuronidase/arylsulfatase treatment, suggesting the occurrence of phase-II conjugates [4]. In addition, STC forms moderately strong complexes with human serum albumin, which may influence detectability in serum [5]. Analogous to the well-described carry-over of AFB₁, a controlled *in vivo* transfer study with four dairy cows was conducted. Animals received an oral STC bolus for 14 days (60 μ g/day). Serum samples were collected at baseline and repeatedly throughout supplementation and the wash-out phase. Serum was analysed for STC and selected metabolites by LC–MS/MS after enzymatic hydrolysis with β -glucuronidase. Preliminary findings indicate no detectable STC in milk samples (LOD 0.01 ng/mL) and only sporadic trace levels of STC in urine (2/8 samples; 0.18–0.20 ng/mL). Whilst a comparable low-dose AFB₁ exposure would result in measurable AFM₁ in milk (5.76 \pm 0.97 ng/kg) [6], STC appears to undergo a different metabolism. Data on STC blood levels still remain to be evaluated.

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3.31 P31 - Elucidating the effect of deoxynivalenol on the activity profile of topoisomerase inhibitor SN-38

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While the general population might cope with minor mycotoxin exposure without adverse health effects, patients, and especially colorectal cancer patients under chemotherapy, are more vulnerable. The gut, especially the colon, is an organ with a high mycotoxin exposure, and its barrier function is often decreased during chemotherapy. The *Fusarium* toxin deoxynivalenol (DON) is among the most prevalent contaminants in food and feed. Besides its own toxicological effects, DON has previously been reported to exacerbate the genotoxic effects of possible co-contaminants as well as anticancer medications (1). Irinotecan, a topoisomerase inhibitor, is frequently administered during the therapy of various types of cancer, including colorectal cancer. During therapy, gastrointestinal side effects, such as diarrhea, induced by the active metabolite SN-38, are frequently observed (2). Thus, even slight modifications of its activity profile might be of great concern. Consequently, the aim of the study was to determine whether DON also modulates SN-38 activity and potential side effects, as well as to investigate the underlying mechanisms.

To that end, DON was combined with irinotecan's active metabolite, SN-38, in HCT116 human colon cancer cells and HCEC-1CT human colon epithelial cells. Combinatory cell viability measurements (CellTiter Blue assay) showed additive effects after 24 and 72 h. While DON indeed amplified SN-38-induced DNA damage (measured by γ H2AX staining and DNA-formamidopyrimidine glycosylase-modified alkaline comet assay) in HCT116 cells after 4 h of treatment, after 20 h of recovery, this DON-induced increase was not observed in the comet assay anymore (although γ H2AX remained elevated). Similarly, DON increased SN-38-induced replication stress (detected by loss of 5-ethynyl-2'-deoxyuridine incorporation and increased phosphorylation of replication protein A) after 4 h, which was lost after 20 h of recovery. Initial transcriptomic analysis of the combined effect of DON and SN-38 in HCT116 cells revealed changes in cytoskeletal, ribosomal, and proliferative gene sets. Interestingly, in HCEC-1CT cells γ H2AX signal exacerbation by DON was more pronounced after 20 h of recovery than without, suggesting a potential difference in response between cancerous and non-cancerous cells.

Consequently, DON has the potential to enhance SN-38 activity, although with varying sensitivity across cell types, which might contribute to the severity of gastrointestinal side effects of irinotecan anticancer therapy.

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3.32 P32 - Mycotoxin Profile of Maize in AP Vojvodina, Serbia and the Republic of Srpska, BIH: Influence of 2024 Weather Conditions

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In 2024, maize production in AP Vojvodina (Serbia) and the Republic of Srpska (BIH) was marked by pronounced weather fluctuations, raising concerns about mycotoxin occurrence. This study investigated the profile and concentration of 21 regulated and emerging mycotoxins in 360 maize samples collected from major agricultural regions in both countries using a modified and validated liquid chromatography tandem mass spectrometry method (LC-MS/MS) (Hofmann and Scheibner, 2021). Comprehensive weather data from both countries, including temperature and precipitation, were analyzed to evaluate their influence on mycotoxin contamination levels. Aflatoxins (Afs) were highly prevalent, particularly in AP Vojvodina (85.9%) compared to the Republic of Srpska (44.2%). The aflatoxin precursor sterigmatocystin was detected less frequently (11.1% and 6.1%, respectively), while ochratoxin A occurred in ~38% of samples in both regions. Among *Alternaria* toxins, alternariol monomethyl ether (AME) was the most prevalent, occurring in 97.6% of samples from AP Vojvodina and 86.7% from the Republic of Srpska, while alternariol and tentoxin were rarely detected (<10% in both countries). Fumonisin (B1 and/or B2) showed particularly high occurrence, contaminating 98.9% of AP Vojvodina and 74.1% of Republic of Srpska maize samples. In addition to fumonisins, moniliformin (MON) was frequently detected, with incidence rates of 78.8% and 82.3%, respectively. On the other hand, deoxynivalenol and zearalenol were found at low rates, with no more than 20% and 10%, respectively, in either region. Among trichothecenes, fusarenon X was the predominant type B compound, with a markedly higher prevalence in AP Vojvodina (91.1%) than in the Republic of Srpska (66.3%). In contrast, type A trichothecenes (T-2 and/or HT-2) were less prevalent in AP Vojvodina (32.9%) compared to the Republic of Srpska (45.9%). The incidence of ergot alkaloids (ergocorine, ergocristine, ergocryptine, and ergosine) reached 58.9% in AP Vojvodina and 32.6% in the Republic of Srpska. Elevated temperatures and markedly reduced precipitation (June–August) in both regions were strongly associated with increased contamination levels, particularly AFs, fumonisins, MON, and AME. The especially dry conditions in AP Vojvodina, notably in August, corresponded to higher contamination levels compared to the Republic of Srpska. These results confirm the significant influence of climatic factors on mycotoxin occurrence and emphasize the need for integrated monitoring and adaptive management strategies to safeguard food safety under changing climate conditions.

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3.33 P33 - Mycotoxin exposure following an eight-week vegan or meat-rich dietary intervention: A randomized-controlled trial in healthy individuals

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Individuals following a vegan diet (VD) might be exposed to higher dietary intakes of mycotoxins due to their increased consumption of typical mycotoxin-containing plant-foods, including grains, nuts, seeds, and non-dairy milk- and yoghurt alternatives. Few studies specifically addressed this topic and contrasted urinary mycotoxin excretion in vegans in comparison to omnivores. We present results from a secondary data analysis of an eight-week isocaloric vegan dietary intervention (1). In a two-arm, randomized-controlled trial, healthy adults were randomly allocated to either a meat-rich diet (MD) or a VD for 8 consecutive weeks. Foods of animal origin were not permitted on the VD; participants in the MD group were asked to consume at least 150 g of meat daily. Prior to the random group assignment, all participants underwent a one-week run-in phase which included a mixed diet as per the dietary recommendations of the German Nutrition Society (DGE). The SAFIA mycotoxin screening assay (SAFIA, SAFIA Technologies GmbH, Berlin, Germany) was used to analyze morning urine concentrations of the following six mycotoxins (d.l. = detection limit): Aflatoxin (d.l. 0.21 µg/l), Deoxynivalenol (d.l. 2.79 µg/l), Fumonisin (d.l. 3.55 µg/l), Ochratoxin (d.l. 0.11 µg/l), T2 (Trichothecene) (d.l. 1.46 µg/l), Zearalenone (d.l. 0.52 µg/l). Crude and creatinine-adjusted mycotoxin concentrations were analyzed. Food intake was based on two sets of weighed food diaries (3 days each). Spearman rank-order correlations were run to examine potential associations between urinary mycotoxin concentrations and food intakes. Non-parametric tests (Wilcoxon rank-sum test, Mann–Whitney-U-Test) and parametric tests (paired and unpaired two-tailed t-tests) were used to examine within-group (pre- vs. post-intervention) and between-group differences (at baseline vs. at the end of the study). We also ran MMRM (mixed model repeated measures) with the time-diet-interaction as a “fixed effects” and without participant level random effects. The study sample included $n = 63$ participants. Energy intake was not different between the groups. After 8 weeks, creatinine-adjusted Deoxynivalenol concentrations were higher in the VD group (6.99 (8.36) vs. 2.85 (5.94) µg/g in the MD group; $p = 0.008$) whereas T2 concentrations were higher in the MD group (5.15 (5.81) vs. 3.36 (3.83) µg/g in the VD group; $p = 0.033$). Higher creatinine-adjusted Ochratoxin A (0.40 (0.38) vs. 0.21 (0.28) µg/g) and Zearalenone concentrations (1.41 (1.43) vs. 0.53 (1.01) µg/g) were observed in the VD group at week 8 (with p -values: 0.012 and 0.001, respectively). MMRM results pointed in the same direction with a significant diet-time-interaction term for Ochratoxin A ($p = 0.004$) and Zearalenone ($p = 0.006$). Moderate positive correlations (Spearman’s ρ ranging from 0.321 to 0.478) were found between vegetable intake and all mycotoxins (except for T2). A weak association between fish/seafood intake and T2 excretion was observed ($\rho: 0.291$). Plant yoghurt intake was associated with almost all mycotoxins (except for T2). Mycotoxin exposure pattern for vegans appears different from omnivores, requiring attention from a food safety and toxicology perspective.

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3.34 P34 - Apocarotenoids and fusaric acid: potential metabolic cross-talk during flax - *Fusarium* interaction

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Fusaric acid (FA), a secondary metabolite produced by various *Fusarium* species, is widely recognized as a phytotoxin contributing to disease development in plants (1). Despite extensive studies on FA toxicity and biosynthesis, the ecological and host-derived factors regulating its production during plant infection remain poorly understood. Increasing evidence indicates that plant - pathogen interactions involve bidirectional chemical communication, in which both partners exchange signaling molecules that influence metabolism and defense responses (2). Among plant metabolites, carotenoid-derived apocarotenoids have recently emerged as regulators of stress signaling and plant immunity (3). However, their potential role in modulating fungal mycotoxin production has not been explored.

In this study, we investigated the relationship between FA and apocarotenoid signaling during the interaction between flax (*Linum usitatissimum* L.) and *Fusarium* f. sp. *lini* using complementary experimental approaches combining plant treatments, fungal analyses, and metabolic profiling. Plants were treated with fusaric acid to evaluate host physiological and molecular responses, including reactive oxygen species (ROS) accumulation, expression of pathogenesis-related (PR) and carotenoid cleavage dioxygenases (CCD) genes, abscisic acid (ABA) levels, and changes in apocarotenoid content. In parallel experiments, plants were treated with volatile apocarotenoids (α - and β -ionone), and the effects on fungal colonization and mycotoxin production were assessed. Additionally, inhibitors of carotenoid cleavage dioxygenases (CCDs) were applied to investigate the involvement of endogenous apocarotenoid biosynthesis in regulating FA accumulation.

Exogenous FA application triggered pronounced stress responses in flax, including changes in levels of mRNA transcripts of studied genes and ROS, accompanied by alterations in ABA and apocarotenoid profiles, suggesting that FA influences host metabolic signaling pathways beyond its direct phytotoxic effects. Exogenous treatment of infected plants with ionones led to increase in FA accumulation in shoots at later timepoint, despite fungal amounts remaining comparable to control plants. These findings suggest that plant responses induced by volatile ionones can indirectly reshape production of FA by *Foln* in flax, separating toxin production from fungal growth dynamics. Manipulation of endogenous apocarotenoid biosynthesis using CCD inhibitors suggested a potential involvement of carotenoid-derived signaling pathways in the regulation of FA levels, indicating that host metabolic status may contribute to mycotoxin accumulation during infection.

Overall, our results suggest that FA and plant apocarotenoids may participate in bidirectional chemical signaling during plant - fungus interactions. FA may therefore act not only as a virulence factor but also as part of a broader signaling network linking fungal metabolism with host defense responses. These findings further suggest that plant-derived volatile apocarotenoids may be involved in systemic plant responses associated with mycotoxin accumulation. Understanding these interactions may provide new perspectives on the ecological regulation and potential management of mycotoxin accumulation in plant - pathogen systems.

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3.35 P35 - Sharper Eyes on Ergot: Advancing Detection in Wheat

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Background & Objective: Ergot alkaloids (EAs), produced by *Claviceps purpurea*, are a major food safety concern in wheat because their occurrence can be highly variable across grain samples. Accurate detection is essential for reliable monitoring, regulatory compliance, and effective management of ergot-affected grain. This study aimed to advance EA detection in wheat by comparing enzyme-linked immunosorbent assay (ELISA) screening with UHPLC-MS/MS quantification and by developing an epimer-specific UHPLC-HRMS method to improve analytical sensitivity, specificity, and confidence in EA profiling.

Methods: The performance of a commercial ELISA kit was evaluated using standards and in-house reference materials. Cross-reactivity was assessed based on IC₅₀ responses of individual alkaloids relative to ergotamine. Spiked solvent and wheat matrix samples were analyzed by ELISA and UHPLC-MS/MS, and method agreement was evaluated using regression analysis and Bland-Altman plots.

Results: ELISA showed a strong relationship with UHPLC-MS/MS but underestimated total EA concentrations, indicating that screening results are influenced by cross-reactivity differences among individual alkaloids and matrix-related effects. Cross-reactivity varied across EA compounds, with certain alkaloids producing stronger immunoassay responses than others. The developed UHPLC-HRMS method achieved strong specificity, sensitivity, mass accuracy, and epimer separation, allowing more reliable quantification and detailed profiling of major EAs in wheat.

Conclusion: ELISA is useful for rapid screening of ergot alkaloids in wheat; however, confirmatory chromatographic methods are essential for accurate quantification. The developed UHPLC-HRMS method provides sharper analytical insight by improving sensitivity, specificity, and epimer-specific detection, supporting more reliable wheat monitoring, food safety assessment, and management of ergot-affected grain.

Keywords: Ergot alkaloids; wheat; ELISA; UHPLC-MS/MS; UHPLC-HRMS; epimer-specific quantification; food safety

3.36 P36 - Comparison of ELISA, UHPLC-MS/MS, and Development of UHPLC-HRMS Method for Ergot Alkaloid Quantification in Wheat

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Background & Objective: Wheat is critical to Canada's economy, contributing approximately \$10.2 billion annually, with 80% of wheat exported. However, ergot contamination caused by *Claviceps purpurea* poses a significant food safety risk due to the presence of toxic ergot alkaloids (EAs). This study aimed to evaluate the performance of an enzyme-linked immunosorbent assay (ELISA) for EA detection compared to ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Additionally, an ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) method was developed and validated to improve the accuracy and sensitivity of EA detection.

Methods: The ELISA kit was validated using standard and in-house reference materials. CR was determined as the ratio of IC₅₀ values of individual alkaloids to ergotamine. Spiked solvent and wheat matrix were analyzed using ELISA and UHPLC-MS/MS, followed by regression analysis and Bland-Altman plots. A UHPLC-HRMS method was developed and validated for enhanced EA quantification.

Results: ELISA showed a strong correlation with UHPLC-MS/MS ($r=0.8793$) but underestimated total EA concentrations by a factor of two. CR varied widely, with ergometrine exhibiting the highest CR in solvent and wheat matrix, indicating substantial matrix effects. UHPLC-HRMS provided superior resolution, sensitivity, and mass accuracy, enabling comprehensive EA profiling and identification of previously undetected alkaloids.

Conclusion: While ELISA offers a rapid screening approach, its accuracy is highly influenced by matrix effects and CR variability. The validated UHPLC-HRMS method enhanced analytical capabilities by providing high sensitivity and specificity, complementing UHPLC-MS/MS for regulatory compliance and improved food safety in wheat monitoring, ensuring more reliable and comprehensive analysis.

Keywords: ELISA, UHPLC-MS/MS, Ergot Alkaloids, Cross Reactivity

3.37 P37 - Aflatoxins in maize and milk in Serbia: Multi-year trends and implications for food and feed safety

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Over the past two decades, Serbia has experienced pronounced climate change, characterized by rising air temperatures, more frequent heatwaves, and prolonged droughts. The country is warming approximately 60% faster than the global average. These climatic shifts have significantly influenced aflatoxins occurrence, particularly in maize, Serbia's most widely cultivated crop and a major export commodity.

A multi-year analysis of maize (2010–2025) revealed highly variable but increasingly frequent aflatoxins contamination. In certain years (2010–2011, 2014, 2016, 2018–2020), aflatoxins were absent or detected in less than 20% of samples, whereas in drought-affected seasons (2012, 2013, 2015, 2017, 2021–2025) contamination rates ranged from 30% to 84%. In these nine separate years, the highest contamination frequencies were consistently associated with severe summer droughts and elevated temperatures. Alarmingly, during four consecutive years (2021–2025), aflatoxins were constantly detected in maize, with some samples from 2024 and 2025 exceeding 100 µg/kg. Considering maize's central role as both a staple food and feed commodity, contamination directly affects products along the maize-based food and feed chain, with milk serving as a primary route of human exposure.

The carry-over of aflatoxin B1 from contaminated feed into milk as aflatoxin M1 (AFM1) has posed a persistent public health concern in Serbia since 2012. During periods of intensified maize contamination, AFM1 was consistently detected in milk, with up to 95% of samples contaminated and over 70% exceeding the European Union maximum level of 0.05 µg/kg in some years. Risk assessments consistently identify infants and young children as the most vulnerable group due to high milk consumption relative to body weight. This widespread contamination has also affected dairy products, disrupted export opportunities, caused economic losses, and generated consumer confusion due to information gaps and regulatory misalignment with EU standards during the period 2013–2026.

Beyond food and feed safety concerns, recurrent aflatoxins contamination represents a major economic challenge, affecting maize exports, livestock production, and the sustainability of the dairy sector. The increasing frequency of extreme climatic events suggests that aflatoxins contamination may become the "new normal" in Serbia. Addressing this challenge requires strengthened monitoring systems, climate-adaptive agricultural practices, harmonization of regulatory frameworks, and a multidisciplinary approach integrating research, risk assessment, and stakeholder education across the food and feed chain.

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3.38 P38 - Zearalenone modulates the expression of apoptotic markers in ovarian cancer cells

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Introduction

Zearalenone (ZEN) is a widespread foodborne *Fusarium* spp. mycotoxin with estrogenic activity. However, its effects on ovarian cancer (OC) cells and the underlying molecular mechanism remain incompletely understood. It was presented that mycotoxins may induce oxidative stress and apoptosis in various cell lines. Altered expression of Bax, Bcl-2, caspase-9 (Casp9) and caspase-3 (Casp3) and their cleaved forms is crucial for the regulation of apoptosis, as these proteins are among the main components of the mitochondrial apoptotic pathway.

Materials and methods

Two ovarian cancer cell lines (OVCAR3 and SKOV3) were used as *in vitro* models of ovarian cancer. Cells were treated with concentrations of 10 or 30 μ M for 24 hours and harvested for analysis. Gene expression was analyzed using RT-qPCR, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene. Relative expression levels were calculated using the $\Delta\Delta$ Ct method. Protein expression was assessed by Western blotting, and densitometric analysis was performed using Image Lab software (Bio-Rad Laboratories). Statistical significance was evaluated using one-way ANOVA. A p-value < 0.05 was considered statistically significant (GraphPad Prism software).

Results

Treatment with ZEN led to induction of apoptosis in ovarian cancer cells, as evidenced by molecular markers of the mitochondrial pathway. Specifically, ZEN increased the gene expression of pro-apoptotic *Bax* and *Casp9* in both cell lines. Moreover, it also increased the expression of the cleaved forms of caspase-9 and caspase-3 at the protein level, with simultaneously decreasing the levels of anti-apoptotic Bcl2.

Conclusion

The results showed that ZEN modulates key regulators of the mitochondrial apoptotic pathway in ovarian cancer cells

Funding

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3.39 P39 - The role of flavonoids in maize (*Zea mays* L.) fungal resistance

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Maize (*Zea mays* L.) is an important crop worldwide, particularly in low-income regions such as Sub-Saharan Africa, where it is a staple food and important source of income. However, maize yields are increasingly threatened by several abiotic and biotic stresses, including heat and drought as well as infections with phytopathogenic fungi. Species of the genera *Fusarium* and *Aspergillus* are of interest as they are frequently able to produce hazardous mycotoxins. Mycotoxins not only impair plant health but pose serious risks to human and animal health. The occurrence of fungal infections and mycotoxin contamination is especially high in eastern Kenya, where hot and dry climatic conditions promote fungal growth and toxin production. To mitigate fungal infection and the associated mycotoxin contamination, the SolFOOD project investigates the role of flavonoids in maize resistance. The direct antifungal effects of selected flavonoids were assessed using microplate assays, in which four flavonoid derivatives were tested at different concentrations and their efficacy was compared with that of the active substance tebuconazole. In addition, flavonoid contents were analysed in maize varieties exhibiting different levels of resistance to *Aspergillus flavus* and *Fusarium* spp. using near-infrared spectroscopy and liquid chromatography–mass spectrometry.

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3.40 P40 - Stability of regulated and emerging mycotoxins in water slurry mixtures of corn and peanut matrices under different storage conditions

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At WFSR, most routine mycotoxin analyses employ slurry mixing during sample preparation; however, the stability of mycotoxins in aqueous slurry matrices has not been systematically assessed. This study investigated the stability of a selected range of mycotoxins in water slurry mixtures prepared from corn and peanut matrices, with the aim of determining whether immediate analysis is required or whether slurry samples can be stored without compromising analyte integrity. Slurry mixtures were prepared by homogenising each matrix with water, then stored under room temperature, refrigerated, and frozen conditions for multiple time intervals.

Corn containing naturally occurring mycotoxins, and peanut materials were spiked, homogenised with water (1:2 w/w), and subsampled to assess homogeneity (1, 2). Remaining aliquots were stored at -80 °C (reference temp. point), -20 °C, 4 °C, and 20 °C, and analysed at defined intervals from 2 hours to 168 days. All analyses were performed using the QuEChERS-based SOP-2145 method (3). Homogeneity assessment showed that the peanut material was sufficiently homogeneous for all analytes. In the corn material, homogeneity could not be conclusively demonstrated for fumonisin B1 and enniatin B due to elevated within-sample variability, despite no measurable between-sample differences. For all other analytes, homogeneity criteria were met.

Stability evaluations revealed that corn slurry samples stored at -20 °C maintained excellent stability across all analytes, with most results remaining within $\pm 10\%$ of the -80 °C reference. Refrigeration (4 °C) provided acceptable short-term stability (up to 72–168 hours), whereas room temperature storage (20 °C) resulted in noticeable degradation for several toxins. DON, fumonisins, enniatins, beauvericin, CPA, and alternariol exhibited strong stability across conditions. In another experiment at WFSR, T-2 toxin showed rapid enzymatic conversion to HT-2 toxin in corn slurry, with approximately half of the T-2 toxin converted within 2.5 minutes of adding water, preventing meaningful stability assessment for this analyte. It was also investigated by Sujin Lee et al. (4), who described 84–86% conversion within 15 min in the presence of crude protein extracts from corn and brown rice.

In peanut slurry, most analytes demonstrated robust stability across all temperatures. Aflatoxins, fumonisins, DON, ZEN, OTA, alternariol, the enniatin group, and beauvericin showed recoveries largely $\geq 90\%$ relative to the reference. Minor reductions were observed for AFB2 and T-2 toxin at 20 °C, but no significant degradation trends were detected across the wider analyte panel. Refrigerated storage preserved stability for several days, whereas room temperature storage led to small but measurable decline for T-2 toxin over extended periods.

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3.41 P41 - Hepatic metabolism of naturally occurring ergot alkaloids: Insights from human and porcine liver microsomes

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Grains and their processed products are frequently contaminated with ergot alkaloids due to the infection with *Claviceps species*. Over the last decades increasing infections have been detected in European countries. According to the *European Food Safety Authority* children of all age groups are potentially at health risk following the consumption of rye products [1]. Therefore, new lower limits for the total amount of ergot alkaloids have been in force since 2024 [2].

While the pharmacological and physiological effects of ergot alkaloids have been well documented, comprehensive characterization of their biotransformation products remains limited. In contrast, research on metabolism has been conducted for semi-synthetic derivatives of ergot alkaloids. For example, dihydroergotamine is used as a therapeutic agent due to the enhanced vasoconstrictive and neuropharmacological properties compared to the parent compound. Available data indicate extensive hepatic metabolism of these drugs, primarily mediated by cytochrome P450 [3]. Regarding naturally occurring ergot alkaloids cell culture studies demonstrate that human intestinal and liver cell lines are capable of metabolizing ergot alkaloids to a spectrum of hydroxylated metabolites. The position of the hydroxy groups could be narrowed down to the proline moiety using HPLC-FTMS [4].

In order to gain further insight into the metabolism of naturally occurring ergot alkaloids, detailed analyses were performed on ergometrine, ergocristine and ergosine and their corresponding isomers. Human and porcine liver microsomes were used, and analyses were carried out by high-resolution mass spectrometry to enable further structural elucidation of the resulting metabolites.

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3.42 P42 - The effect of sterigmatocystin on the glutathione redox system and lipid peroxidation in broiler chicken with selenium supplementation

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Sterigmatocystin (STC) is a relatively understudied mycotoxin whose relevance to feed safety is expected to increase as a consequence of climate change. Warmer climatic conditions favor the proliferation of STC-producing molds such as *Aspergillus versicolor* and *Aspergillus nidulans*. Its effects in avian species are not yet well characterized, and in broiler chickens in particular, there is a notable lack of information regarding its impact on lipid peroxidation, the antioxidant defense system, and membrane fatty acid composition. Although the antioxidant properties of selenium (Se) are well established, its potential protective role against STC exposure has not yet been investigated. This research may provide new insights into whether Se supplementation could serve as an effective nutritional strategy to mitigate oxidative damage induced by mycotoxins, including STC. The study aimed to investigate the interaction between STC and Se on the fatty acid composition of membrane lipids and lipid peroxidation processes in the liver of 21-day-old broiler chickens. *In vivo* study was performed in a short-term (5 days) feeding trial with 2 mg STC/kg feed, using Se supplementation in the form of hydroxy selenomethionine with and without STC in two doses: 0.3 mg/kg, 0.5 mg/kg. Markers of the glutathione system and lipid peroxidation in the liver were determined using biochemical methods, including the measurement of reduced glutathione levels, glutathione peroxidase activity, malondialdehyde, and conjugated dienes and trienes levels. Furthermore, the fatty acid composition of hepatic membrane phospholipids lipids was determined. Based on our results there was significant differences in the amount and activity of the elements of the glutathione redox system as a function of the exposure time as compared to the untreated group, which suggest that the well-known oxidative stress-inducing effect of the STC. Se exhibits a protective role against STC-induced oxidative stress and lipid peroxidation, with potential dose-dependent effects. Fatty acid profile also changed as effect of STC, and its combination with Se.

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3.43 P43 - The effect of acerola and wild rose extracts on mould growth and mycotoxin production

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In recent years, research into natural methods for protection against mould and its toxins has become increasingly important. This is due to the frequent occurrence of mould and the growing threat of mycotoxin contamination in food products (1).

Extracts from plants with high antifungal activity, such as acerola (*Malpighia emarginata*/*Malpighia glabra* L.) and wild rose (*Rosa canina* L.), are a promising area of research. The aim of this study was evaluate the effectiveness of acerola and wild rose extracts in inhibiting mould growth and reducing mycotoxin production.

Acerola, also known as Barbados cherry, is characterised by an exceptionally high vitamin C content and other bioactive compounds, such as polyphenols, carotenoids and organic acids. With its powerful antioxidant and anti-inflammatory properties, acerola extracts has also demonstrated antifungal activity (2).

Wild rose, especially its fruit, is a rich source of vitamins (especially vitamin C), flavonoids, organic acids and tannins, which have a wide range of antioxidant, antibacterial and antifungal properties (3). Rosehip extracts can inhibit the growth of various strains mould and may also contribute to reducing the mycotoxins levels, which pose a serious threat to human and animal health.

The studies evaluated the effects of acerola and wild rose extracts on the growth of moulds of the genus *Aspergillus* and the level of mycotoxins produced by adding the extracts to the culture media on which the moulds were growing.

The study aims to test the potential of plant extracts as natural preventive agents in protecting food against mould and mycotoxins. This is an important step towards the development of alternative, environmentally friendly methods of mould protection that can be used in the food, pharmaceutical and agricultural industries. Thanks to their antifungal and antioxidant properties, acerola and wild rose extracts can be a safe and effective alternative to chemical fungicides, offering potential benefits for both human health and environmental protection.

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3.44 P44 - Comparative lung toxicity of sterigmatocystin and its precursors

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Mycotoxins are well known as dietary toxicants, and toxicological studies are therefore conducted in this way. However, mycotoxin-producing fungi are also highly prevalent in indoor environments, particularly in moisture-damaged buildings, where fungal bioaerosols may represent a relevant inhalation exposure route. Among indoor-associated species, *Aspergillus versicolor* and *Aspergillus nidulans* produce sterigmatocystin (STE), a possible human carcinogen (IARC group 2B) that is also an intermediate in the biosynthetic pathway of Aflatoxin B1 (AFB1)[1,2]. During the synthesis of STE, several compounds are produced and co-accumulate with STE, such as Versicolorin A (VerA), Averantin (AVN) and Averufin (AVF)[3]. Despite the detection of STE and related precursors in air samples, data on their respiratory toxicity remain limited.

In this study, we compared the cytotoxic and genotoxic effects of STE, Versicolorin A (VerA), Averantin (AVN) and Averufin (AVF), in human bronchial (H358) and alveolar (A549) epithelial cell lines as in vitro models of inhalation exposure. AFB1 was also used as control, since STE and VerA share structural similarities related to their mutagenicity. Cells were exposed for 24 h to concentrations ranging from 0.1 nM to 30 µM. Genotoxicity was assessed by γH2AX quantification by immunofluorescence using high-content analysis, and cell viability was measured in parallel using a luminescence-based enzymatic assay quantifying cellular ATP content.

All compounds induced dose-dependent effects, with marked differences between mycotoxins and cell lines. H358 cells were globally more sensitive to VerA, whereas A549 cells showed higher sensitivity to STE. The lowest concentration inducing γH2AX in H358 cells was 0.1 µM for AFB1, 3 nM for VerA and 30 nM for STE while A549 cells showed an increase of γH2AX starting at 0.3 µM for AFB1 and VerA, and 10 nM for STE. These results indicate higher sensitivity of H358 cells to the tested toxins compared to A549 cells. AFB1 and STE exhibited predominantly genotoxic profiles, with cytotoxicity occurring at DNA-damaging concentrations. VerA showed a mixed profile, with cytotoxic effects occurring at lowest concentrations than genotoxic effects, whereas AVN and AVF mainly affected viability without significant DNA damage. These compound-specific responses, together with differences between bronchial and alveolar cells, suggest distinct modes of action along the aflatoxin biosynthetic pathway and differences in metabolic competence. Consistently, differential sensitivity to benzo[a]pyrene, a compound requiring CYP-mediated metabolic activation, was observed only in H358 cells, supporting cell line-specific metabolic capacities likely linked to CYP expression.

Overall, these results indicate that aflatoxin biosynthetic intermediates are highly toxic to respiratory epithelial cells, even at very low concentrations, and exhibit distinct toxicity profiles. They support the health relevance of assessing the toxic effects following inhalation exposure to these mycotoxins in indoor environments and highlight the need for further studies to better characterize their respiratory toxicological effects.

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3.45 P45 - Correlations Between Maize Chemical Composition and *Fusarium* Mycotoxin Occurrence

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Fusarium moulds are among the most important pathogenic moulds that can produce mycotoxins in maize crops, with mycotoxin production influenced by the genetic predisposition, interactions with changing climate patterns, and potentially by the chemical composition of the crop (1,2). The aim of this study was to investigate the correlations between the chemical composition of maize and the occurrence of *Fusarium* mycotoxins. For this purpose, 67 maize samples were collected in October and November from Croatian family-owned farms prior to storage. The samples were analyzed for 15 *Fusarium* mycotoxins, including zearalenone (ZEA) and its metabolites ZEA-14-*O*- β -glucoside and ZEA-14-sulfate; nivalenol (NIV); deoxynivalenol (DON) and its metabolites (3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside); fumonisin B1 (FB1) and fumonisin B2 (FB2); T-2 and HT-2 toxins; diacetoxyscirpenol (DAS); neosolaniol (NEO); and fusarenon-X (FUS-X), using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The chemical composition analyzed included moisture, ash, fat, protein, sugars, starch, and mineral contents. Spearman's correlation analysis was used to evaluate correlations between individual mycotoxins and chemical parameters of the maize samples. Seven mycotoxins were detected: DON and 15-acetyl-DON, HT-2 and T-2 toxins, fumonisins B1 and B2, and ZEA-14-sulfate. For most detected mycotoxins, a negative correlation with sugar content ($\rho = -0.28$ to -0.37) and a positive correlation with moisture content ($\rho = 0.28$ to 0.35) were observed. High moisture content was shown to be a key factor associated with the occurrence of *Fusarium* mycotoxins, while higher sugar content may be associated with reduced mycotoxin levels, potentially due to changes in osmotic potential, enhanced microbial competition, and/or altered fungal metabolism (3). Fumonisins exhibited a moderate positive correlation with starch content ($\rho = 0.32$, $p \leq 0.01$), while FB1 showed an additional association with fiber content and FB2 with iron content, which may reflect the potential influence of starch-rich endosperm and peripheral grain layers on *Fusarium* spp. colonization (4). HT-2 and T-2 toxins additionally showed negative correlations with calcium content ($\rho = -0.34$, $p \leq 0.01$) and fat content ($\rho = -0.26$, $p = 0.04$), further supporting the role of maize chemical composition in influencing the occurrence of type A trichothecenes, although the underlying mechanisms remain to be clarified.

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3.46 P46 - Disruption of heme biosynthesis as a novel mode of action of enniatins and beauvericin

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One of the major developments in salmon aquafeeds has been the replacement of fish meal and fish oil with plant based ingredients, resulting in significant changes to feed quality and safety. Incorporating plant ingredients into salmon diets has introduced mycotoxins orally to carnivorous marine farmed fish. Although ionophoric mycotoxins such as enniatins are among the most prevalent toxins in modern aquafeeds [1], data on their toxicity to Atlantic salmon remain limited.

Recent attention on mycotoxins in salmonid feeds has led the Institute of Marine Research (IMR) to identify that the emerging, non-regulated mycotoxins beauvericin (BEA) and enniatin B (ENNB) can impair the health of Atlantic salmon (*Salmo salar*) at concentrations already present in commercial feeds [2]. Documented effects include anaemia, impaired bone formation, reduced growth, and liver damage. Our *in vitro* studies further indicated that dysregulation of iron metabolism and subsequent ferroptosis may represent potential toxic mechanisms [3].

A central objective of the MYTOXA project was therefore to investigate the mode of action of these mycotoxins and identify key early toxic events underlying the observed liver pathology and anaemia. By integrating *in silico*, *in vitro*, and *in vivo* approaches, we uncovered a possible alternative mechanism of toxicity. RNA-seq analyses from both *in vivo* and *in vitro* experiments consistently suggested dysregulation of the heme biosynthesis pathway. Initial *in silico* screening identified potential molecular targets for inhibition within this pathway (see Pedroni et al., 2026 MW abstract).

Subsequent *in vitro* assays using human and fish cell lines supported the computational predictions, indicating that disruption of heme biosynthesis enzyme activity constitutes key initiating events in the mode of action of enniatins. Taken together, findings from all three methodological approaches converge on heme biosynthesis disruption as a novel mode of action contributing to enniatin-induced anaemia in Atlantic salmon.

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3.47 P47 - Fungal Infection and Mycotoxin Profile in Spanish Oat Crops: Impact of Meteorological Conditions on *Fusarium* Prevalence

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Oat crops are susceptible to fungal infections influenced by temperature and precipitation. Depending on these conditions, different fungi may dominate, with mycotoxigenic species posing the highest food safety risk. This study surveyed fungal infection and mycotoxin contamination in 91 Spanish oat samples collected from the Ebro Valley during the 2023 and 2024 campaigns, with particular emphasis on *Fusarium* species, and correlated these findings with field meteorological conditions. Samples were surface-disinfected and plated on malachite green agar (MG) and rose bengal chloramphenicol agar (DRBC). *Fusarium* infection was assessed on MG, while other genera were evaluated on DRBC. *Fusarium* isolates were grouped by morphology and mycotoxin production, with a sub-sample of them identified to species level by EF-1 α sequencing. Mycotoxin content was analyzed by UPLC–MS/MS, targeting 23 compounds. *Alternaria* was detected in all samples (mean grain infection 91.5% in 2023 and 98.5% in 2024), followed by *Fusarium* (83.3–95.8% of samples, mean infection 7.7–17.3%). Other detected genera included *Aspergillus*, *Rhizopus*, *Epicoccum*, *Phoma*, *Penicillium*, and *Cladosporium*. Among major *Fusarium* mycotoxin producers, fumonisin producers (*F. proliferatum*, *F. fujikuroi*, *F. verticillioides*) were detected in 46% of samples, followed by deoxynivalenol and zearalenone producers (*F. culmorum*, 24%; *F. pseudograminearum*, 5%; *F. graminearum*, 1%) and T-2 and HT-2 producers (*F. langsethiae*, 24%; *F. sporotrichioides*, 5%). Other notable species included *F. equiseti* (33%), *F. avenaceum* (15%), *F. oxysporum* (12%), *F. tricinctum* (12%), and *F. poae* (10%). *Alternaria* infection was favored by cool and humid conditions, whereas *Fusarium* by low precipitation during early growth and flowering and high temperatures during grain filling. All mycotoxins were below the limits established by European Commission Regulation 2023/915 (1), with DON (75%) and T-2 (70%) being the most frequently detected. Despite the high prevalence of fumonisin-producing *Fusarium* species, only a few samples (3%) were positive for fumonisin B₁. Also, alternariol (10%) and alternariol monomethyl ether (4%) were detected. Fungal infections were influenced by climatic conditions, particularly moisture availability during flowering and grain filling (April–August). *Alternaria* was the most predominant fungal genus in Spanish oat samples, and its associated toxins indicate a potential risk to the food chain.

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3.48 P48 - Consumer Awareness of Mycotoxin Contamination in Food

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Mycotoxins represent a significant but often 'invisible' challenge to food safety. While regulatory limits ensure high standards, consumer behavior—specifically the handling of moldy food and the perception of emerging products like plant-based milk alternatives—plays a crucial role in individual exposure. A study on risk perception aims to bridge the gap between toxicological risk and public awareness.

An online survey (N ≥ 1,000) was conducted among the German-speaking population aged 16 and older with representative quota control for gender, age, education, and federal state.

A central component of the study focuses on mycotoxins, defined as toxic substances released by molds onto food products. Participants are tested on their objective knowledge, including the fact that these toxins are invisible and cannot be rendered harmless through heat treatments like cooking or baking. Furthermore, the study evaluates compliance with recommended behavioral guidelines, relating to food storage and the decision to either discard or partially salvage moldy food items. Given the rising popularity of plant-based diets, a dedicated section examines the risk perception of mycotoxins in vegan milk alternatives like oat and almond drinks. To contextualize these findings, mycotoxins are compared with other substances found in food, such as microplastics, pesticides, and heavy metal residues, in terms of perceived likelihood and severity of adverse health effects. Finally, the research incorporates psychological covariates to better understand consumer responses, including health consciousness, trust in food safety authorities, cognitive reflection and food disgust sensitivity.

3.49 P49 - When mycotoxins taste bitter (and beyond): AI-driven discovery of bitter receptors-fungal indolizidine alkaloids interaction

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Bitter receptors, encoded by TAS2R genes, are expressed not only in the lingual epithelium but also in extraoral tissues, including the gastrointestinal tract. The ectopic expression of bitter receptors, such as those located in the gut, can modulate physiological functions and mediate responses to harmful compounds, including mycotoxins [1, 2].

This study presents a NAMs-based approach, driven by *in silico* analysis, to investigate possible interactions between bitter receptors and a dataset of approximately 3,200 mycotoxins derived from the MycoCentral Database, a comprehensive database curated by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES; <https://www.mycocentral.eu>). First, a general ligand-based model tuned to discover bitter molecules identified 1-indolizidinone, among others, as a potential bitter compound. This molecule belongs to the class of indolizidine alkaloids, a wide group of chemicals produced by plants and fungi, including those from the *Alternaria* genus [3]. Currently, the toxic potential of this largely overlooked group of mycotoxins and the mechanisms underlying their possible harmful effects remain poorly understood.

This scenario prompted us to further analyse the interaction between the 25 human TAS2R receptor and a list of fungal 1-indolizidinone analogues, including swainsonine, slaframine, curvulamine, curindolizine, bipolamine A, bipolamine G, and 1-hydroxyindolizidine, through an AI-supported 3D molecular modelling pipeline. This additional computational analysis revealed that some members of this class of fungal metabolites exhibit potentially stable interactions with multiple TAS2Rs. This confirmed the potential bitterness of certain indolizidine alkaloids but also provided a plausible mechanism of action to investigate further in future dedicated analysis.

These results eventually underscore the utility of computational methods to enhance the understanding of mycotoxin toxicology.

Keywords: computational toxicology, *Alternaria*, bitter receptors, NAMs, molecular modelling

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3.50 P50 - Moldy foods: how to predict mycotoxin production and migration in foods?

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Edible food waste amounted to 3.8 million tons in France in 2023, a third of which was produced by households (1). Such food waste is partly due to mold contamination. While some consumers may directly discard moldy foods, others may consume them as is, or after trimming to limit food waste. These practices are common since molds are visible. However, they can be a concern for food safety as certain fungal species produce toxic mycotoxins that may migrate into the product. Mycotoxin production and migration are highly dependent on fungal species and strains and influenced by the extent of fungal growth (i.e. thallus size), storage temperature, mycotoxin chemical properties, as well as food composition and structure (2). Although fungal contaminations and regulated mycotoxins are frequently monitored along the production chain, their occurrence at the household level is still limited and consumer behavior towards moldy foods is not well known. By integrating consumer behaviour, fungal growth, and spatial toxin distribution into a microbiological risk assessment (MRA) strategy, the goal of our project is to identify unsafe practices and provide consumers with guidelines to reduce food waste while ensuring their safety.

Participatory science was first used to collect moldy foods, identify mycotoxin-producing molds and determine consumer behaviour regarding moldy food handling strategies (discard or consume without removal or with trimming at varying distances from the visible fungal lesion). Multiple food-fungal species associations were then selected for mycotoxin migration studies. Here, strawberry jam contaminated by a mycotoxin producing *Penicillium verrucosum* strain was used as an example. *P. verrucosum* UBOCC 109221 was isolated from a high sugar content food and produces two regulated mycotoxins: citrinin (CIT) and ochratoxin A (OTA) (3). Strawberry jam is a favorable medium for fungal growth and mycotoxin migration, except at high sugar contents, and widely consumed in France (4). *P. verrucosum* was point inoculated onto the surface of jams and stored at temperatures to mimic consumer storage conditions (8 and 20 °C). Thallus diameter was monitored and OTA and CIT concentrations were quantified according to depth when the mold colony reached 1, 2, 3, 4 and 5 cm in diameter. Based on the 3D migration strategy we previously developed (3), jam cylinders were cut and mycotoxin concentrations were quantified at different depths.

Toxin production rate was estimated according to mold size, and migration into the jam was characterized by a migration coefficient based on Fick's law and the finite element method. These parameters enabled the integration of the dataset into a spatial migration model to simulate mycotoxin concentration at any point in the product depending on visible thallus size, distance from the thallus and storage temperature. Coupled with jam consumption data from the latest French food consumption survey (4), we will determine the extent of mycotoxin exposure occurring among the French population, which will also be based on multiple behavioural scenarios regarding food storage and handling strategies.

To our knowledge, this is the first time that such a multidisciplinary strategy integrating consumer behavioural and consumption data with fungal growth, mycotoxin production and spatial migration modelling is applied.

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3.51 P51 - Effect of Non-Thermal Plasma Exposure on the Mycotoxin Profile of *Aspergillus niger*

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Preventing *Aspergillus niger* contamination is critical for maintaining both food safety and economic sustainability in food production systems. Antifungal interventions must not only inhibit fungal growth but also prevent or reduce mycotoxin formation (1).

Non-thermal plasma (NTP) has demonstrated antifungal efficacy, including inactivating *A. niger*, a common spoilage fungus in the food industry (Kulisova et al., 2024a)(2). However, its influence on fungal secondary metabolism and mycotoxin production remains insufficiently understood.

The aim of this study was to evaluate the effect of NTP treatment on the mycotoxin profile of *A. niger* DBM 4054 cultivated on agar and in liquid medium. Cultures were grown for 24 or 48 h prior to 90 min NTP exposure and subsequently incubated for an additional 1–72 h, enabling comparison with untreated controls, which were cultivated up to 96 h. Mycotoxins were extracted using a modified QuEChERS protocol and quantified by UPLC-MS/MS. Twenty-three mycotoxins were monitored.

Three mycotoxins were detected: fumonisin B₂ (FB₂), ochratoxin A (OTA), and ochratoxin B (OTB). During 24 h cultivation, no mycotoxin production was observed on agar and only low levels of OTA were detected in liquid medium, regardless of NTP treatment. In liquid medium, OTB appeared only after ≥72 h of total cultivation. Across both matrices, cultures treated with NTP after 48 h and harvested following extended post-treatment incubation generally showed lower OTA and OTB accumulation compared to untreated controls of comparable total age.

Overall, NTP treatment did not induce new mycotoxin production and was associated with reduced accumulation of OTA and OTB. These findings suggest that non-thermal plasma represents a promising food-safe intervention strategy capable of limiting fungal toxin production without stimulating adverse metabolic responses.

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3.52 P52 - Developing a new methodology for the detection of mycotoxins in human breast milk

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Mycotoxins are toxic secondary metabolites produced by certain fungi that can enter the food chain through contaminated plant- and animal-derived foods. Increasing evidence shows that some mycotoxins can be detected in human breast milk following maternal dietary exposure reflecting both environmental contamination and the transfer of parent toxins across biological barriers (1). Although concentrations are typically low, early-life exposure is of particular concern because infants have immature detoxification systems, high food intake relative to body weight, and amplified vulnerability to toxic, immunomodulatory, and carcinogenic effects (2). Aflatoxin M1 (AFM1), ochratoxin A (OTA), deoxynivalenol (DON), T-2/HT-2 toxins and zearalenone (ZEN) were investigated using a newly developed and validated analytical method. The method was applied to breast milk samples collected from a group of 70 lactating mothers enrolled in a national biomonitoring program. The findings indicate low detection frequencies and generally low exposure levels, supporting the conclusion that maternal exposure does not compromise the well-established benefits of breastfeeding.

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3.53 P53 - Phenolic Compounds in the Control of *Fusarium* Growth and Toxigenicity

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Fungi of the *Fusarium* genus are globally distributed plant pathogens responsible for significant yield losses in many economically important crops. In addition to reducing agricultural productivity, these fungi produce a wide range of mycotoxins that pose serious risks to plant, animal, and human health. The increasing resistance of pathogens to synthetic fungicides, together with growing concerns about their environmental impact, highlights the urgent need for sustainable and environmentally friendly disease management strategies. Naturally occurring phenolic compounds, widely present in plants, are considered promising candidates due to their antimicrobial properties and potential ability to inhibit fungal growth and mycotoxin biosynthesis.

In this study, we investigated the effects of selected phenolic compounds (ferulic acid, p-coumaric acid, vanillin, and vitexin) on the growth and development of *Fusarium oxysporum* f. sp. *lini* and *Fusarium culmorum*. We further assessed their impact on the production of fusaric acid and beauvericin (in *F. oxysporum* f. sp. *lini*) as well as deoxynivalenol and its acetylated derivatives (in *F. culmorum*). Additionally, we analyzed the expression levels of key genes involved in the biosynthetic pathways of these mycotoxins. Our results indicate that phenolic compounds can inhibit mycelial growth, affect fungal development and modulate mycotoxin production.

In conclusion, this study highlights the potential of plant-derived phenolic compounds as sustainable and eco-friendly alternatives for limiting *Fusarium* proliferation and reducing its toxigenic capacity. By modulating fungal growth and interfering with mycotoxin biosynthesis pathways, these natural metabolites may represent promising components of integrated crop protection strategies.

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3.54 P54 - Zero-Waste Valorization of Pomegranate Peel for the Control of *Aspergillus flavus* Growth and Aflatoxin B₁ Production

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Aflatoxin B₁ (AFB₁), produced by aflatoxigenic strains of *Aspergillus flavus*, remains one of the most hazardous mycotoxins threatening food and feed safety worldwide. This study proposes an integrated biorefinery strategy based on pomegranate (*Punica granatum* L.) peel, an agro-industrial by-product rich in polyphenols, to simultaneously suppress fungal growth and reduce AFB₁ contamination.

Lyophilized ultrasound-assisted ethanolic pomegranate peel extracts (PPE), rich in phenolic content (155.81 mg/g GAE), were evaluated for their antifungal and antitoxigenic activities. The agar dilution method was applied using Potato Dextrose Agar (PDA) and Maize Agar Medium (MAM), with PPE incorporated at concentrations of 0.5, 1, 3, 5 and 10 mg/mL. PPE significantly inhibited *A. flavus* mycelial growth, achieving a 73.42% reduction at 10 mg/mL after 10 days of incubation on PDA. Moreover, PPE reduced AFB₁ production by 81.33% on MAM, demonstrating a strong inhibitory effect on both fungal development and toxin biosynthesis.

To further valorize the residual biomass, the fibrous press cake obtained after polyphenol extraction was investigated for its ability to remove AFB₁ via biosorption. Fiber characterization showed an increase in insoluble fiber content from 14.04±0.20 to 44.09±0.37 g/100 g (dry basis), with enrichment in cellulose, hemicellulose, and lignin, key components for toxin binding. Under simulated gastrointestinal conditions, the press cake exhibited high AFB₁ adsorption efficiency, reaching 76.86% at pH 3 and 79.23% at pH 7 at a biosorbent concentration of 30 mg/mL, with concentrations varied between 2 and 35 mg/mL.

Overall, this dual-function biorefinery approach demonstrates that pomegranate peel extracts and their corresponding press cake represent complementary, natural strategies for AFB₁ control, through inhibition of toxin biosynthesis and adsorption of residual toxins, respectively. This comprehensive valorization pathway supports circular economy and zero-waste principles while offering a sustainable solution to a critical food safety challenge. Moreover, these findings provide a foundation for the further research, development and practical application of pomegranate peel-based preparations in food and feed safety management.

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3.55 P55 - Mycotoxin Contamination in Peanuts (*Arachis hypogaea L.*) at Post-harvest Stage in Eastern Ethiopia

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Peanuts are highly susceptible to contamination by toxigenic fungi and the associated mycotoxins during both pre- and post-harvest stages. However, in Ethiopia, data on the occurrence and levels of major mycotoxins in peanuts remain limited. Moreover, since 2016, there have been no published studies reporting the mycotoxin contamination status of peanuts in eastern Ethiopia, a region known for significant peanut production in the country. Thus, this study aimed to investigate the current occurrence and concentration of mycotoxins in post-harvest peanut samples (N = 54) collected from January to March 2023, from the 2022 harvest season, from Babile and Fedis districts, as well as the surrounding areas of Harar town. All samples were analyzed for different mycotoxins, including aflatoxins (AFB1, AFB2, AFG1, and AFG2), fumonisins (FB1, FB2, and FB3), ochratoxin A (OTA), and sterigmatocystin (STERIG) using LC-MS/MS. The results revealed that 61% of the samples were contaminated with at least one mycotoxin, with up to six different mycotoxins detected in a single sample. The highest contamination was observed in samples from the Harar area, where nine mycotoxins were detected. Samples from Babile and Fedis were also contaminated with up to seven mycotoxins. Notably, the highest concentrations of AFB1 (4222 µg/kg), AFB2 (3497 µg/kg), and OTA (178 µg/kg) were detected in Harar samples. Babile samples showed the highest levels of AFG1 (3136 µg/kg) and AFB1 (2483 µg/kg), while Fedis samples had the highest AFG2 concentration (106 µg/kg). Fumonisins (FB1, FB2, and FB3) were detected at relatively low levels (2.2–37.0 µg/kg). This is the first report confirming the presence of fumonisins, OTA, and STERIG in Ethiopian peanuts. Alarmingly, 19% and 17% of the samples exceeded the European Commission and U.S. regulatory limits for total AFs (4.0 and 20.0 µg/kg, respectively). The findings highlight substantial contamination with multiple mycotoxins, particularly AFs and OTA, posing a significant public health risk. The study underscores the urgent need for comprehensive food safety interventions across Ethiopia's peanut production and supply chain.

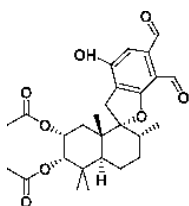
Keywords: Mycotoxin prevalence; LC-MS/MS; Peanuts; Post-harvest; Health risks; Ethiopia.

3.56 P56 - Isolation and structure elucidation of biologically active secondary metabolites from *Stachybotrys* spp

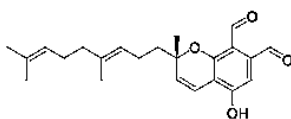
Mehrsima Montaser, Florian Hübner, Hans-Ulrich Humpf, Svetlana Kalinina

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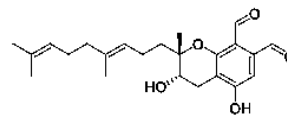
Fungi detected in feed and food products can produce mycotoxins - so-called toxic secondary metabolites, that pose significant health risks to both animals and humans. Despite their harmful effects, filamentous fungi are also recognized as an important source of structurally diverse natural products (NPs) with potential biological activity. However, the secondary metabolites (SMs) of many fungal species remain poorly characterized, including those of the toxigenic black mold *Stachybotrys* spp. To address this knowledge gap, the present study focuses on the understudied species *Stachybotrys bisbyi* and *S. microspora*, aiming to comprehensively investigate their metabolic profiles, identify potentially novel secondary metabolites, and evaluate their toxicity and biological activity. One of the main issues with both strains is their weak possibility to grow. In order to get an access to the novel secondary metabolites as well as to get enough biomass for the isolation it was important to optimize their media for cultivation. Overcoming the cultivation challenges using a food-based medium (potato cellulose oat milk agar (PCOMA), composed of tap water, oat milk, agar, cellulose, and potato infusion powder), we develop an untargeted approach employing high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) to monitor potential secondary metabolites production. This led to the selection of three metabolite groups for isolation: phenylspirodrimanes (PSDs), stachybotrychromenes, and triprenyl phenols (SMTPs) (3, 4). Following isolation and structural elucidation, we successfully obtained single-crystal X-ray diffraction data for one of the principal metabolites, stachybotrydial, thereby providing the first confirmation of its absolute configuration. The isolated metabolites subsequently served as starting materials for semi-synthetic modifications aimed at generating biologically active derivatives (5). The synthesized compounds were then evaluated for their biological activity and cytotoxic potential.



PSDs



Stachybotrychromenes



Pre-SMTP

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3.57 P57 - Contribution of terrestrial processes in reducing environmental Deoxynivalenol levels

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This study provides an overview of the literature on the main terrestrial processes influencing environmental levels of mycotoxins, using deoxynivalenol (DON) as an example. Levels of DON in agricultural soils are 100–1,000 times lower ($> 30 \mu\text{g kg}^{-1}$), compared to levels in crops, food and feed items (mg kg^{-1}). This indicates that in soils certain processes may occur that reduce DON levels. DON enter to soil mainly through mobilization with water from contaminated crops. In soils, DON interacts with soil particles with almost no sorption. However, minor chemical modifications, such as acylation (e.g. 15-ADON) or additional OH groups (e.g. nivalenol), can influence mycotoxin-soil interactions. Carbonyl functions (15-ADON) are decisive for adsorption to clay particles, while the aromatic character (e.g. zearalenone) supports sorption to soil organic matter². Therefore, the mobility of mycotoxins in soils depends on the chemistry of the mycotoxins interacting with the structure and chemistry of the soil. By interactions with the soil microbiome, DON is biotransformed to *keto*-DON and *epi*-DON³, independently of the soil type. The O-deacetylation of 15-ADON and 4-acetyl nivalenol (fusarenon X) is a rapid (within hours), microbial-driven conversion that leads to deoxynivalenol and nivalenol, respectively². The microbial dissipation of DON is improved by a higher biomass and activity, and a narrow fungi-to-bacteria ratio³. The uptake of DON by plant roots is estimated by only 20%. However, it is known to elicit various responses in the plant, including the accumulation of metabolites associated with the phenylpropanoid pathway, which is known to be involved in both biotic and abiotic stress responses. On the other hand, the role of DON as a virulence factor in this situation is not clear, indicating a complex interaction between the two players⁴. Consequently, DON levels in environmental compartments can be interpreted as the net result of entry and dissipation processes mediated through the soil rather than the soil acting as a source of them⁵

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3.58 P58 - Hidden Estrogenic Burden in Finished Feed: Co-Occurrence of Phytoestrogens and Mycoestrogens in European Livestock Diets

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Phytoestrogens (PE) are naturally occurring plant secondary metabolites that structurally resemble 17 β -estradiol, allowing them to interact with estrogen receptors, preferentially to ER β , and exert estrogenic or anti-estrogenic activity. They are abundant in leguminous plants such as clover and alfalfa, as well as in soybeans and cereal grains, and comprise major classes including isoflavones, lignans, and coumestans. In farm animals, dietary exposure to phytoestrogens can modulate reproductive performance, endocrine regulation, and growth, with outcomes influenced by dose, species, and physiological status. Concurrently, the mycoestrogen zearalenone (ZEN) frequently co-occurs with PE in finished feed, raising concerns about additive or synergistic estrogenic effects in exposed livestock (1).

In our dsm-firmenich World Mycotoxin Survey 2025, 180 finished feed samples originating from Europe were analyzed with the multi-toxin LC-MS/MS method (2), revealing a high prevalence of the phytoestrogens daidzein and genistein and their glycosides, daidzin and genistin (96–99%). Mean concentrations were substantial, ranging from 33,400 to 51,700 $\mu\text{g}/\text{kg}$ for the glycosides and 4,300 to 5,700 $\mu\text{g}/\text{kg}$ for the aglycones. Zearalenone (ZEN) was detected in 86% of the samples, with an average concentration of 44 $\mu\text{g}/\text{kg}$ and a maximum level of 468 $\mu\text{g}/\text{kg}$ in one sample. Additionally, alternariol (AOH), a mycotoxin reported to exhibit weak *in vitro* estrogenic activity (3), was detected in 49% of all samples, with a mean concentration of 11 $\mu\text{g}/\text{kg}$. Despite its comparatively lower potency, its frequent occurrence contributes to the overall burden of estrogenic compounds in finished feed and may enhance the cumulative endocrine-disrupting potential in exposed animals. In pig feed samples, phytoestrogens were detected in 100% of cases, with mean concentrations reaching up to 35,700 $\mu\text{g}/\text{kg}$. At the same time, ZEN was detected in 84% of the samples with an average concentration of 20 $\mu\text{g}/\text{kg}$. This is particularly relevant because pigs are highly sensitive to endocrine-active compounds and are especially vulnerable to estrogenic effects.

These findings highlight the need for studies investigating potential synergistic interactions among these substance classes to evaluate a possible effect on the sensitive hormone system, to support evidence-based risk assessment by regulatory authorities.

This work was created within a research project of the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI).

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3.59 P59 - From *Alternaria* extract to alterperyleneol: Discovery of an immunosuppressive mycotoxin targeting NF- κ B

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Fungi of the genus *Alternaria* can produce a broad spectrum of structurally diverse secondary metabolites, of which the toxic compounds are referred to as *Alternaria* mycotoxins. Some of these compounds are frequently detected in food at relatively high concentrations and can cause a broad range of adverse effects, posing a potential risk to human health¹. Previously, a natural toxin mixture obtained by cultivating a strain of *Alternaria alternata* on rice was shown to exert genotoxic and anti-estrogenic effects *in vitro*². Building on these findings, the present study aimed to investigate the immunomodulatory properties of this complex extract, identify the single mycotoxins responsible, and elucidate the underlying molecular mechanisms, with a focus on the NF- κ B signaling pathway.

First the crude extract was evaluated for its immunosuppressive potential using an NF- κ B reporter assay in LPS-stimulated THP-1 monocytes, which revealed strong suppression of NF- κ B activation. To identify the compound(s) responsible for these effects, toxicity-guided fractionation using RP-HPLC was performed. Individual fractions were screened for their activity, leading to the identification of a fraction containing high concentrations of the perylene quinone alterperyleneol (ALTP) as particularly active. ALTP was then tested for its ability to inhibit the NF- κ B pathway and modulate the expression and secretion of pro-inflammatory cytokines IL-6, IL-8, and TNF- α at both mRNA and protein levels through qRT-PCR and ELISA respectively. Considering that ingestion is the main route of exposure to mycotoxins, cytokine modulation was also assessed in intestinal epithelial Caco-2 and HCEC-1CT cells. Cell viability was monitored in parallel using the CellTiter Blue[®] assay to ensure that observed effects were not due to cytotoxicity. Modulation of key proteins of the NF- κ B pathway was further examined in THP-1 monocytes via Western blot and immunofluorescence microscopy.

ALTP potently suppressed LPS-induced NF- κ B activation starting at 1 μ M and effectively reduced cytokine expression and secretion in both immune and intestinal epithelial cells. TLR4 protein levels remained unchanged, whereas phosphorylated IKK α / β , NF- κ B p65, and phosphorylated NF- κ B p65 were downregulated as shown by Western blot analyses. Immunofluorescence microscopy revealed upregulation of the inhibitory protein I κ B α and decreased phosphorylation of its inhibitory form, indicating stabilization of the I κ B–NF- κ B complex and impaired nuclear translocation of NF- κ B.

In conclusion, these findings identified ALTP as a potent immunosuppressive *Alternaria* mycotoxin that interferes with central regulatory proteins of the NF- κ B pathway. Its activity in immune and intestinal cells underscores the need for further studies to assess the risks associated with dietary exposure to this emerging toxin.

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3.60 P60 - Ergot Alkaloids in Italian Cereals and Cereal-Based Products: A Five-Year Monitoring Study (2020–2025)

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Ergot alkaloids (EAs) are naturally occurring tryptophan-derived mycotoxins produced by fungi of the genus *Claviceps* that infect cereals and replace the grain with sclerotia known as ergot (1). Changing climatic patterns may create favourable conditions for fungal infection of cereals, increasing contamination risks along the cereal supply chain (2).

EAs, occurring as biologically active -ine forms and their -inine epimers, pose significant food safety concerns due to their vasoconstrictive and neurotoxic effects (1).

Commission Regulation (EU) 2023/915, established harmonised maximum levels for EAs in specific cereal commodities, reinforcing monitoring activities across the EU (3).

This study investigates the occurrence and concentration levels of EAs in a set of 312 samples collected in Italy between 2020 and 2025 as part of official control activities, including both raw materials and cereal milling products. The largest food category consisted of cereal-based raw materials ($n = 171$), including wheat flours, barley, oats, rice and spelt. Additional samples comprised cereal-based baby foods ($n=46$), cereal-derived milling products ($n=47$) and rye samples ($n=48$).

A validated LC–MS/MS method was applied for the determination of 12 ergot alkaloids after QuEChERS (Z-Sep/C18) purification. The limit of quantification (LOQ) was 2 $\mu\text{g}/\text{kg}$ for each analyte.

Overall, 55 samples (17.6%) showed quantifiable levels of EAs ($>\text{LOQ}$). Rye represented the most critical cereal, with the highest contamination levels (maximum 5412 $\mu\text{g}/\text{kg}$; mean 584 $\mu\text{g}/\text{kg}$). Seven rye flour samples exceeded the maximum level of 250 $\mu\text{g}/\text{kg}$ established by Regulation (EU) 2023/915. Ergocristine and ergocristinine were the predominant alkaloids across all samples.

Among wheat and other cereal flours, the highest concentrations detected were 64 and 130 $\mu\text{g}/\text{kg}$, both remaining below the applicable regulatory limit of 150 $\mu\text{g}/\text{kg}$. Processed cereal-based products showed lower levels (maximum 39 $\mu\text{g}/\text{kg}$; mean 10 $\mu\text{g}/\text{kg}$). All baby food samples were below the LOQ except one (2.4 $\mu\text{g}/\text{kg}$).

A temporal evaluation highlighted an increasing frequency of samples with quantifiable EAs, rising from 10% in 2020 to 23% in 2025. This trend likely reflects strengthened monitoring activities following regulatory updates and possibly environmental factors influencing fungal development.

The results confirm rye as the most susceptible cereal matrix and demonstrate the effectiveness of targeted monitoring in ensuring regulatory compliance within the Italian market.

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Keywords: Ergot Alkaloids; LC-MS/MS; cereals; monitoring study

3.61 P61 - Comparative Intestinal Permeability and Molecular Responses to Major Aquafeed Mycotoxins in RTgutGC Cells

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The incorporation of cereal-based ingredients into aquafeeds has increased the risk of mycotoxin contamination, potentially affecting fish and human health and industry productivity (1). However, the mechanisms underlying mycotoxin absorption and their impact on fish intestinal barrier remain poorly understood (2). Intestinal epithelial cell models have emerged as powerful tools for investigating *in vitro* nutrient and contaminant transport, providing a greener, ethical, reproducible, and high-throughput alternative to *in vivo* experimentation. Among these, the RTgutGC cell line represents the first established fish intestinal epithelial cell model (3).

RTgutGC was used to assess intestinal transport (apical to basolateral direction) and following transcriptional responses after exposure to aflatoxin B1 (AFB1), fumonisin B1 (FB1), enniatin B (ENNB) and enniatin B1 (ENNB1). Cells were seeded in permeable inserts (62500 cells/cm², 12-well plates, 28-day differentiation) and exposed to environmentally relevant concentrations, 1.281, 0.554, 0.625, 0.612 µM, respectively. Cell viability and barrier integrity were assessed through neutral red (NR) assay and transepithelial electrical resistance (TEER) measurements. Mycotoxin translocation across the epithelial layer was quantified at different time intervals (3, 12 and 24 h) by LC-MS/MS to calculate the toxins' Apparent Permeability (P_{app}). After the transport assay, qPCR was used to analyse gene expression of key markers involved in epithelial barrier function, immune and stress response (ZO-1, CLDN3, IALP, iMUC, IL-8, IL-1β, TLR3, TNFα and HSP70).

P_{app} varied among the tested mycotoxins. FB1 showed no detectable translocation across the barrier. AFB1 (P_{app}= 5.09x10⁻⁰⁵ cm/s) exhibited rapid initial transport (t=3h), followed by a slower increase (t=12h). At the final sampling point, the receiver concentration decreased, potentially indicating efflux transport. ENNB (P_{app}= 1.00x10⁻⁰⁵ cm/s) and ENNB1 (P_{app}= 1.58x10⁻⁰⁶ cm/s) displayed different transport rates. ENNB showed high translocation up to t=12h followed by a reduction of the transport rate, whereas ENNB1 showed a linear translocation, reaching a maximum at t=24h. All mycotoxins induce similar regulation of immune and stress response genes, indicating activation of inflammatory and protective pathways. In contrast, barrier-related genes were differentially regulated, with FB1 slightly downregulating tight-junction protein genes. Overall, these findings provide mechanistic insights into mycotoxin-specific intestinal absorption and barrier disruption in fish and support improved risk assessment and mitigation strategies for aquafeed contaminants. Also underline the relevance of fish intestinal *in vitro* cell models as predictive tools for assessing mycotoxin bioavailability and toxicity in aquaculture.

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3.62 P62 - Long-term monitoring of mycotoxins in barley, malt and beer: trends, transfer and analytical challenges

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Mycotoxins are a well-known food safety concern in cereal-based products, including brewing raw materials and beer. This study builds on our previous pilot work on mycotoxins in barley, malt and beer and extends it to long-term monitoring, evaluation of changes over time, and assessment of mycotoxin transfer during the malting and brewing process.

From 2020 to 2025, a total of 2,446 samples of barley (n = 926), malt (n = 801) and commercially available beers (n = 719) were analyzed for major regulated and emerging mycotoxins. These included aflatoxin B1, deoxynivalenol (DON), its acetylated forms and the modified form deoxynivalenol-3-glucoside (DON-3G), zearalenone and its metabolites, fumonisins, T-2 and HT-2 toxins, and ochratoxin A. Sample preparation was based on QuEChERS extraction and immunoaffinity clean-up, followed by UPLC-MS/MS analysis.

Mycotoxins were detected in all studied matrices. Concentrations were generally in the µg/kg range for barley and malt and in the ng/L range for beer. Most samples met current regulatory limits, but clear differences between years and between matrices were observed. These differences reflect the influence of climate, raw material quality and processing conditions. Special attention was given to the presence of DON and DON-3G during malting and brewing, showing that modified mycotoxins should be considered in exposure and risk assessment.

The long-term cooperation between the Research Institute of Brewing and Malting and Fianovis highlights the importance of reliable analytical methods, appropriate reference standards and continuous monitoring. The results improve the understanding of mycotoxin behavior in brewing and malting and support effective consumer protection and food safety.

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3.63 P63 - Organic vs. Conventional and Jars vs. Pouches: A Comparison of Patulin incidence in Apple-based Baby Food

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Baby fruit-based purees represent a common component of the diet of infants and young children. These products typically contain 60–100% fruit and are available in a wide range of flavour varieties. Because apples are relatively inexpensive, shelf-stable, and technologically suitable compared with many other fruits, they are commonly used as the primary fruit ingredient. In multi-fruit purées, apple generally constitutes at least 30% of the total fruit content. Due to patulin (PAT) mutagenic, neurotoxic, immunotoxic, genotoxic, and nephrotoxic effects, PAT is regulated by Commission Regulation (EU) No. 2023/915 as amended.

This study systematically assessed the occurrence of the mycotoxin PAT in apple-based baby puree, with particular attention to contamination patterns associated with production systems (organic versus conventional) and packaging formats (glass jars versus pouches). PAT was isolated using molecularly imprinted polymer columns and subsequently quantified by High-Performance Liquid Chromatography coupled with diode-array detection. Statistical analyses were performed to characterise variability in PAT levels across the evaluated product categories.

The global dataset comprised 200 samples of apple puree. PAT was detected in 173 samples (87%) at concentrations above the limit of detection (0.3 ng/g), while 84 samples (42%) exceeded the limit of quantification (1.0 ng/g). Using the middle-bound approach for descriptive statistics, the mean PAT concentration was 1.03 ± 1.17 ng/g, with a median value of 0.50 ng/g. The 90th percentile reached 2.08 ng/g, and the maximum measured concentration was 11.01 ng/g.

The resulting data provide a detailed perspective on the PAT-related safety of apple-based baby food, thereby informing consumer risk awareness and reinforcing the need for manufacturers to minimise PAT contamination throughout the production process. The detail results of this study will be presented at the 47th Mycotoxin Workshop.

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3.64 P64 - Zearalenone and ochratoxin A induced hormonal imbalance in female reproduction

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Hormones play a fundamental role in women's health by regulating physiological processes throughout life, including puberty, pregnancy, lactation, and menopause. Estradiol and progesterone are key ovarian steroid hormones responsible for menstrual cycle regulation, fertility, and maintaining pregnancy, while prolactin is essential for lactation and modulates reproductive and metabolic functions. Cortisol, a major glucocorticoid hormone, plays a central role in the stress response and interacts with reproductive hormones via the hypothalamic–pituitary–adrenal axis (1,2). Disruption of these hormonal pathways may contribute to endocrine and reproductive disorders.

Exposure to xenoestrogens, including the mycotoxins such as ochratoxin A (OTA) and zearalenone (ZEN), is increasingly recognised as a factor affecting endocrine homeostasis (1,2). These compounds originate from contaminated food and environmental sources and interfering with hormonal signalling. OTA has been linked to stress-related pathways (3), while ZEN acts as a potent mycoestrogen capable of disrupting oestrogen and progesterone signalling, potentially impairing fertility and reproductive health (4,5).

A total of 234 samples were collected from women who had been diagnosed with an endocrine disorders. The following were examined: 78 blood samples, 78 urine samples and 78 placenta samples. OTA was quantified using HPLC-MS/MS and OchraPrep (immunoaffinity) columns, while ZEN was detected using HPLC-MS/MS with DZT columns. OTA was detected in the samples at concentrations up to <0.27 ppb, while ZEN reached a maximum concentration of ≤ 0.072 ppb.

Urine samples (n=78 samples) were collected from women after childbirth. Cortisol, prolactin, progesterone, and estradiol concentrations were determined using electrochemiluminescence assays with two-point calibration, prolactin was measured using a sandwich assay. The obtained hormone concentrations remained within physiologically plausible ranges, indicating preserved endocrine homeostasis. The concentrations were as follows: cortisol 6.10-20.40 ng/mL, estradiol 0.03-0.36 ng/mL, progesterone 0.58-0.88 ng/mL, and prolactin 0.05-3.1 ng/mL.

The observed inter-individual variability may reflect physiological changes associated with the postpartum period, as well as adaptive endocrine responses potentially modulated by chronic exposure to hormonally like environmental contaminants, including mycotoxins. Preliminary results suggest a potential link between exposure to hormonally active mycotoxins and endocrine disorders in women, highlighting the importance of these studies and the need for their further research.

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3.65 P65 - Airborne *Aspergillus* species in a Zoological Garden: diversity, cytotoxic effects and mycotoxin production

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Introduction: Bioaerosols in zoological gardens may pose a health risk to both animals and staff due to the presence of filamentous fungi capable of producing toxic secondary metabolites.

Objective: This study aimed to examine the diversity of airborne *Aspergillus* species in a zoological garden and to assess their cytotoxic activity and mycotoxin production.

Material and methods: Air samples were collected using an impact method with a MAS-100 air sampler on Sabouraud agar. Fungal isolates were identified based on morphological characteristics supported by ITS rDNA sequencing. Cytotoxicity of selected strains was evaluated using the MTT assay, while mycotoxin profiles were determined by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).

Results: A wide range of *Aspergillus* species was detected, representing the sections Nigri, Circumdati, Fumigati, Flavi, and Nidulantes. The dominant species were *A. fumigatus* and *A. niger*, whereas other taxa occurred less frequently. Seventeen isolates, including *A. fumigatus*, *A. flavus*, and *A. tamarii*, were subjected to toxicological analysis. All tested isolates exhibited cytotoxic effects, with several showing moderate to high toxicity. Gliotoxin was the only mycotoxin detected and was produced exclusively by *A. fumigatus* strains.

Conclusion: No clear correlation was observed between cytotoxicity levels and gliotoxin presence, suggesting the involvement of other secondary metabolites. These findings indicate that air in zoological gardens may serve as a significant source of exposure to cytotoxic fungal metabolites, emphasizing the importance of regular air quality monitoring.

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3.66 P66 - Combined estrogenic effects of zearalenone with other myco-, xeno-, and phytoestrogens on *Tg(vtg1:mCherry)* zebrafish embryos

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Zearalenone (ZEN) – a mycotoxin produced by *Fusarium* molds – is a frequent contaminant in maize and other cereals. Due to its ability to activate estrogen receptors (ERs), ZEN is a well-known non-steroidal xenoestrogen. Previous *in vitro* studies demonstrated the combined estrogenic effects of ZEN with other xeno- and phytoestrogens (1–3). The estrogen sensitive liver transgenic zebrafish model (*Tg(vtg1:mCherry)*) is highly suitable for the investigation of the estrogenic impacts of xenobiotics, where the magnitude of ER activation is concentration-dependent and directly proportional to the signal intensity and the size of the affected fluorescent area (4). In the current study, we examined the combined impacts of ZEN with zearalenone-14-sulfate (Z14S), zearalenone-14-glucuronide (Z14GA), alternariol (AOH), alternariol-3-sulfate (A3S), bisphenol A (BPA) and bisphenol S (BPS), glyphosate (GLY) and its metabolite aminomethylphosphonic acid (AMPA), and phytoestrogens daidzein (DAI) and genistein (GEN). In our model, ZEN showed marked estrogenic effect at 100 µg/L (314 nM) concentration. The combined impacts of myco-, xeno-, and phytoestrogens were tested at 250 nM, 750 nM and 2 µM concentrations, where most of the compounds examined did not show estrogenic effects alone. Z14GA did not affect significantly, while lower levels of Z14S decreased the estrogenic action of ZEN. AOH induced no or only slight changes; however, A3S antagonized the impact of ZEN at each concentration examined. Co-treatment with BPS increased ER activation, while BPA caused elevation only at lower levels. AMPA and DAI did not change significantly, but GLY and GEN gradually increased the estrogenic effect of ZEN. The combined impacts of estradiol with Z14S and A3S were also tested: 250 nM of Z14S decreased and 2 µM of A3S increased the estradiol-induced effect. Our results demonstrate the complex modulatory impacts of myco-, xeno-, and phytoestrogens on the estrogenic effect of ZEN, where besides the receptorial, other (e.g., toxicokinetic) interactions seem to be involved.

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3.67 P67 - Potential of cold atmospheric plasma for mitigation of mycotoxin contamination in milk thistle

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Milk thistle (*Silybum marianum*) is among the most widely used medicinal plants in hepatoprotective phytotherapy. Preparations rich in silymarin flavonolignans are globally consumed as dietary supplements as well as herbal medicinal products (1,2,3). However, recent studies indicate that milk thistle is frequently contaminated with mycotoxins, especially trichothecenes, zearalenone, *Alternaria* mycotoxins, enniatins and beauvericin (2,4). Of particular concern are HT-2 and T-2 toxins together with *Alternaria* mycotoxins, as regular consumption of milk thistle preparations may result in biologically relevant dietary exposure (4). Effective post-harvest mitigation strategies capable of reducing contamination while preserving bioactive constituents are therefore needed. Cold atmospheric plasma (CAP) has emerged as a promising non-thermal decontamination technology based on the generation of reactive oxygen and nitrogen species, UV radiation, and charged particles capable of microbial inactivation and chemical transformation of small molecules as mycotoxins (5). The present study investigates the effects of CAP treatment on selected mycotoxins relevant to milk thistle contamination. Two plasma systems generating (i) oxygen-based reactive species (CAP-O) and (ii) nitrogen-based reactive species (CAP-N) were applied to pure mycotoxin standards. Samples were analysed using ultra-high performance liquid chromatography and high-resolution tandem mass spectrometry (U-HPLC-HRMS/MS) operated in data-dependent acquisition mode (DDA), enabling comprehensive fragmentation data acquisition without predefined target ions. Preliminary results demonstrate compound-dependent responses to plasma treatment. CAP-O showed higher effectiveness for *Alternaria* mycotoxins, enniatins and beauvericin, whereas CAP-N was more effective for trichothecenes and zearalenone. The highest reductions were observed for enniatins (up to 43%), tenuazonic acid (up to 26%), zearalenone (up to 24%) and deoxynivalenol (up to 20%). The poster will further present plasma-induced transformation patterns of individual mycotoxins identified using a molecular networking approach, together with results obtained from CAP treatment of milk thistle seeds, including changes in naturally occurring mycotoxin levels and the stability of silymarin flavonolignans.

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3.68 P68 - Ochratoxin A Quantification in Dry-Cured Ham: results from a preliminary comparison in different laboratories

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Ochratoxin A (OTA) is a toxic, nephrotoxic, and carcinogenic secondary metabolite primarily produced by fungi. Several studies indicate that OTA contamination can occur in dry-cured ham through environmental mold growth (mainly *Penicillium nordicum* and *Aspergillus ochraceus*) on the surface of the product during ripening. Variable levels of OTA contamination in dry-cured ham have been reported, with concentrations sometimes exceeding recommended levels (e.g., up to 160.9 g/kg in some studies) [1]. While often below legal limits, OTA levels in commercial products require strict monitoring, as it is a major mycotoxin risk to human health.

Accordingly, the availability of validated analytical protocols for the accurate quantification of OTA concentration in dry-cured ham is crucial for the implementation of effective control strategies and to mitigate health risks[2].

In this work different analytical procedures, meeting in-house validation criteria, provided significantly variable results for OTA levels in dry-cured ham samples, depending on the conditions applied for the extraction, purification and chromatographic analysis. Even if OTA is currently not subjected to official regulation in EU, these preliminary results underline the analytical challenge to provide reliable results for this matrix, as well as the need for harmonized and specific analytical criteria. Further research and targeted interlaboratory proficiency tests are suggested to deal with this challenge and ensure consistent results.

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3.69 P69 - Moldy foods: how to predict mycotoxin production and migration in foods?

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Food waste occurs worldwide and throughout the entire food chain [1]. While many factors can explain food waste, mold spoilage is frequent at the consumer level [2]. Data on fungal diversity of moldy foods in consumer households is scarce in comparison to earlier stages in the food chain. Molds can not only contaminate and spoil foods but may produce mycotoxins that migrate into the products thus leading to safety concerns for consumers [2]. Recently, we conducted a nationwide citizen science campaign to determine fungal diversity of moldy foods. Over 500 moldy foods were collected, including fruits and vegetables, cheeses, other dairy products, jams, bakery products and dry cured meats. Around ~50% of the identified molds are known mycotoxin-producing species. From this data, relevant food - mycotoxigenic mold associations were determined for migration experiments, one being *A. alternata* - tomato. Tomatoes are one of the most produced vegetables worldwide and *Alternaria* contaminations are frequent [3-4]. To study mycotoxin migration, *Alternaria alternata* was artificially inoculated on tomatoes to evaluate fungal growth and mycotoxin migration using a worst-case scenario approach and conditions mimicking consumer storage (i.e. 8 et 20°C) [5]. Several mycotoxins were simultaneously detected in tomato portions beyond the fungal lesion with tenuazonic acid produced at the highest level and migrating the farthest. To further characterise mycotoxin migration, passive diffusion of *A. alternaria* mycotoxins in tomato medium is currently being studied to calculate the different diffusion coefficients [6]. All data will then be used to construct a predictive model for mycotoxin production and diffusion in tomatoes. This is a first step towards better risk assessment for mycotoxins at the consumer level.

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3.70 P70 - Screening for Aflatoxins in Soybeans and Soybean-Derived Products Available on the German Market by Lateral Flow Assay

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Against the background of a growing shift towards predominantly plant-based diets, soybeans and soy-derived products are increasingly serving as important sources of protein, particularly as alternatives to animal-derived products such as meat and milk. Ensuring the safety of these products is therefore of high relevance for consumer protection. Published data on the occurrence of aflatoxins in soybeans and soy-derived products indicate that aflatoxins may contaminate both raw and fermented products. To date, regulation (EU) 2023/915 has established a specific maximum level (ML) for mycotoxins in soybeans only for ochratoxin A (5 µg/kg). With regard to aflatoxins, no specific ML has been defined for soybeans. Therefore, the MLs established for peanuts and other oilseeds are generally applied, namely 2 µg/kg for aflatoxin B1 (AFB1) and 4 µg/kg for total aflatoxins (sum of B1, B2, G1, and G2). The aim of this study was therefore to further characterize the contamination status with respect to total aflatoxins in soybeans (n=2) and selected soy-derived products, such as soy chunks (n=4), soy flakes (n=2), soy granules (n=1), and soy mince (n=1) available on the German market. Analysis was performed using a commercial lateral flow test (Afia-V AQUA, Waters | VICAM), based on monoclonal antibodies, which enables the detection and quantification of total aflatoxins in a concentration range of 2-300 ng/g. The method employs a water-based extraction without the use of hazardous solvents and provides results within 5 minutes. Prior to sample screening, the test system was evaluated for use with the soybean matrix. First, the availability of aflatoxin-negative samples was ensured. For this purpose, the absence in selected soybean products which had been tested negative in the Afia-V AQUA system was confirmed after reanalysis in a second laboratory (SAFIA Technologies GmbH*) by particle-based multiplex immunoassay. Subsequently, manufacturers claim concerning the limit of detection (LOD) corresponding to the applicable ML for AFB1 in peanuts and other oilseeds was checked for soy products by testing negative samples spiked with AFB1 at levels of 0.5× LOD, 1× LOD, and 2× LOD. Recovery was also checked using spiked sample material. Although the investigations are still ongoing (as of February 2026), the results obtained so far indicate an overall high quality of the products analyzed. Two soy flake products revealed concentrations near to the LOD (2.2–3.1 ng/g), while all other samples showed aflatoxin levels below the LOD of the test system.

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3.71 P71 - *In silico* discovery of potential dehydrogenases for Deoxynivalenol biodegradation

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The conventional method for preventing animal mycotoxicosis consists in the application of feed additives to adsorb mycotoxins, but this is often inefficient due to the toxins' structural diversity. Specifically, deoxynivalenol (DON), a widespread contaminant in cereals, causes major economic losses and severe animal health issues (1). DON primarily compromises intestinal integrity, leading to nutrient malabsorption, vomiting, diarrhea, and feed refusal, thus reducing the animal's performance. Moreover, it exerts strong immunotoxic effects by interfering with protein synthesis and negatively modulating the immune response. Because adsorbents have proven limited mitigation of DON's toxicity, there is an urgent need for advanced alternatives, such as enzymatic biotransformation, to convert DON into less toxic compounds like 3-keto-DON (2).

The computational study aimed to identify and select dehydrogenase candidates for DON degradation, dividing the strategy into data compilation, massive virtual screening, and candidate validation. For the data compilation phase, 21 sequences homologous to the characterized sorbose dehydrogenase from *Ketogulonicigenium vulgare* (3) were selected via BLAST search in GenBank for the initial screening pool. The virtual screening involved predicting the three-dimensional structures of these enzymes using AlphaFold, followed by extensive molecular modelling of protein flexibility and massive virtual docking of DON to the active site of these protein models. The PQQ cofactor was transferred to the active site during structural alignment. Candidate selection relied on a dual criterion: low binding affinities and structural similarity to a reference orientation of DON compatible with the dehydrogenation reaction. This first screening iteration identified 10 hit proteins. A second iteration was then performed using Hidden Markov Models (HMM) to enrich the pool through a more sensitive search for homologs in UniProt KB, yielding an additional 64 protein sequences as candidates. The combined iterative approach resulted in 74 total dehydrogenase candidates. The validation phase confirmed that the selected hits exhibited catalytically competent binding modes of DON.

The iterative computational pipeline efficiently identified a large and diverse pool of dehydrogenase candidates — notably from *Acidobacteria*, *Devosia*, *Ketogulonicigenium*, and *Pseudogluconobacter* genera — demonstrating high binding affinity and precise catalytic orientations for DON biotransformation. These validated computational targets are highly valuable for guiding subsequent experimental research into biotechnological detoxification products.

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3.72 P72 - Synergistic Effects of Fumonisin B1 and Polystyrene Microplastics on Porcine Renal and Ovarian Explants

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Environmental contamination by mycotoxins and microplastics poses risks to animal and human health, especially because this challenge is compounded by a lack of specific regulations for chemical mixtures and a significant gap in the scientific understanding of their combined toxicological effects (1; 3; 4). Using porcine tissue explants (3Rs principle) (2), this study evaluated the toxicological effects of Fumonisin B1 (FB1) and polystyrene microplastics (MP-PS) on kidney and ovary models. Cortical renal and ovarian explants from hybrid pigs were cultured in supplemented DMEM and exposed to four groups: control, FB1 (2 μ M), MP-PS (0.4 mg/mL), and Mix (FB1+MP-PS). Incubation lasted 4h (kidney) and 12h (ovary). Tissues were processed for histopathology (lesion scores) and morphometry (Bowman's capsule and space). Data were analyzed by ANOVA/Tukey or Kruskal-Wallis/Dunn ($p < 0.05$). Qualitative analysis revealed that MP-PS and Mix significantly increased renal lesion scores ($p < 0.01$). Quantitatively, MP-PS exposure was the primary driver of structural changes, significantly expanding Bowman's capsule area ($p = 0.032$) and Bowman's space ($p = 0.003$), while FB1 alone induced no changes. Ovarian tissue showed a marginal trend toward increased injury ($p = 0.06$). Our findings demonstrate that co-exposure to FB1 and MP-PS significantly impairs renal integrity, with MP-PS acting as a structural stressor by expanding the urinary space. The observed differential organ sensitivity, where renal tissue proved more vulnerable than ovarian tissue ($p = 0.06$), highlights the complexity of multi-pollutant interactions. These results emphasize the potential of microplastics to exacerbate mycotoxin toxicity through a "carrier effect" similar to other studies findings (5), posing a substantial risk to nephrological and reproductive health. Given the physiological similarities between pigs and humans, this robust *ex vivo* model provides essential translational data for risk assessment and the development of integrated environmental regulations.

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3.73 P73 - Occurrence of mycotoxins in cheese with special cheese toppings

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Cheese is a very popular food in Germany. The German cheese market is known for its wide variety of products, including artisan products, organic products and “farmer-to-consumer” products. There is also a steadily growing number of cheese varieties with extraordinary toppings. This includes the use of plant-based materials as ingredients (like spices, herbs, vegetables) or as floral toppings (like hay, straw, wildflowers, blossom). In this context, it is important to note that various concentrations of mycotoxins have already been detected in various herbs and spices. This leads to the question of whether and to what extent the use of these cheese toppings can directly or indirectly lead to mycotoxin contamination in this type of cheese. The aim of this study is to provide an overview of the presence of mycotoxins in 50 different cheese types with special cheese toppings on the German market by carrying out a comprehensive mycotoxin screening using Enzyme-linked Immunosorbent Assays (ELISA). The cheese samples were made from the surface of the cheese, including the toppings. They were tested for aflatoxin B₁ (AFB₁), sterigmatocystin (STC), mycophenolic acid (MPA) and cyclopiazonic acid (CPA) after preparing the sample accordingly. Preliminary results show that all mycotoxins were detected in varying frequencies and concentrations in the cheese varieties examined. 22 % of the samples analysed tested negative for all mycotoxins examined, while 10 % of samples were positive for all four mycotoxins. A high percentage of samples were tested positive by EIA for AFB₁ (38 %, 0.8 to 2.5 ng/g) and STC (44 %, 0.1 to 9.0 ng/g), but these results require confirmatory analyses, also because toxin levels were near the detection limit of the methods. It was noticeable that most cheese varieties with a pepper topping tested positive for the mycotoxins AFB₁ and STC. In 22 % of samples, MPA was detected in a concentration range of 3.8 to 260 ng/g, while CPA was found in 62 % of samples with values between 100 to 1500 ng/g cheese. Investigations of the most conspicuous samples using Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) to verify the screening results of the ELISA are currently being planned.

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3.74 P74 - Mycotoxin Monitoring in the Context of Climate Change

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Climate change is reshaping fungal communities and secondary metabolite formation in agroecosystems. Increasingly frequent extreme weather events are expected to raise the prevalence and severity of mycotoxin contamination in temperate regions. Long-term, harmonised local monitoring linked to high-resolution weather data is therefore essential to improve field-relevant risk assessment and reduce contamination-related food and feed losses.

In 2024, a long-term field trial was launched at BOKU University in Tulln to uncover temporal trends in agricultural mycotoxin contamination. Twenty wheat varieties with differing yields and resistance levels are cultivated in eight replicates to minimise varietal and local effects; half of the plots receive *Fusarium* provocation using maize plant material. A broad-spectrum “dilute-and-shoot” LC–MS/MS workflow, validated for the simultaneous detection of >1000 agricultural contaminants^{1,2}, enables comprehensive investigation of mycotoxin occurrence under varying environmental conditions, including wide screening for non-regulated fungal metabolites. Mycotoxin data are linked to weather variables (precipitation, temperature, wind, solar radiation) during critical growth phases to identify climate-contamination relationships.

Preliminary results indicate higher deoxynivalenol (DON) concentrations in 2025 compared with 2024. Durum wheat exhibited the highest DON and greater *Fusarium* metabolite diversity; maize pre-cultivation increased metabolite counts and *Fusarium* mycotoxins (e.g., zearalenone, aurofusarin, culmorin). Approximately two weeks around wheat head emergence, a biologically critical phenological window³, mean temperatures in 2025 were significantly higher (two-sample t-test, $p = 2 \times 10^{-23}$), likely favouring *Fusarium* infection. These findings provide a robust starting point for the multi-year trial. Over successive seasons, the resulting evidence base will improve interpretation of weather conditions for predicting in-field mycotoxin contamination and will guide weather-informed decisions that balance effective plant protection with minimising the ecological impact of agricultural practices.

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3.75 P75 - Analysis of mycotoxins and cortisol levels in tissues of wild animals

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In the Polish climate, the most commonly occurring mycotoxins are deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEN), T-2 and HT-2 toxins. Metabolites of these compounds may also be detected in tissues and body fluids, especially α -zearalenol (α -ZOL), β -zearalenol (β -ZOL), α -zearalanol (α -ZAL) and β -zearalanol (β -ZAL). ZEN and its metabolites are known to cause numerous reproductive disorders in both females and males due to their estrogenic activity (1). OTA exhibit hepatotoxic, nephrotoxic, neurotoxic, immunotoxic, mutagenic and teratogenic properties. Toxic effects of DON include diarrhea, feed refusal, weight loss, reproductive, endocrine and digestive disturbances. T-2 and HT-2 toxins may induce loss of appetite, vomiting, weight loss, stomach necrosis, ulcers, hemorrhagic diarrhea, and dermatitis.

Cortisol is one of primary stress hormones. In contrast to adrenaline or noradrenaline, its secretion is delayed and reaches peak levels approximately 20 min after stressor occurrence. Cortisol is synthesized in the adrenal cortex from cholesterol. And plays a key role in maintaining glucose homeostasis by stimulating gluconeogenesis (2).

In this study, tissue samples (liver, kidney and diaphragm) and body fluids (blood and bile) were analyzed for mycotoxins and cortisol concentration. Samples were collected from wild boars, roe deers, red deers, fallow deers and mouflons harvested during seasonal hunts between 2023 and 2025 in the Kuyavian-Pomeranian, Greater Poland and Pomeranian Voivodeships. Both males and females animals were included. The aim of this study was to monitor mycotoxin levels in wild animals tissues, assess cortisol concentration associated with hunting-induced stress, as well as evaluate a potential correlation between mycotoxin exposure and stress hormone levels.. Reason for this hypothesis is based on the structural similarity of ZEN to estrogen enabling it to act as xenoestrogen and potentially interfere with endocrine regulation (3).

Mycotoxins were analysed using HPLC-MS/MS method. Briefly, samples (4g of tissue or 1 ml of fluid) were extracted with acetonitrile:water mixture, with the addition of internal standards and β -glucuronidase. Immunoaffinity columns (DZT-MS Prep for trichothecenes, ZEN and its metabolites; OchraPrep for OTA) were used for sample clean-up.

Cortisol concentrations were determined using electrochemiluminescence method. Samples were incubated with biotinylated antibodies, followed by the addition of streptavidin-coated particles forming cortisol-antibody complex. The electrochemically induced emission of photons was measured using a photomultiplier.

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3.76 P76 - Survey for mycotoxins and plant toxins contamination in medical foods

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Medical foods are specialized products for individuals unable to consume, digest, or metabolize conventional foods due to medical conditions. In the EU, Regulation No. 609/2013 (1) governs foods for vulnerable groups, while contaminant limits are defined under Commission Regulation (EU) 2023/915 (2). Targeted nutritional strategies are crucial for managing conditions such as inborn errors of metabolism, autoimmune disorders, diabetes, and malnutrition. Despite their importance, limited data exist on the safety of medical foods regarding natural toxins. We evaluated 45 commercially available Dutch medical foods, including gluten-free, high-protein, protein-restricted, lactose-free, elemental, low-phenylalanine, and gut-function-specific products, focusing on potential mycotoxin and plant toxin contamination based on the products' ingredients list.

Samples comprised liquid and powdered nutritional products, tube feeding formulas, oral supplements, low-protein diet foods, paediatric nutrition, and products for wound healing or surgical recovery. Analyses included 21 mycotoxins, patulin, cyanogenic glycosides, and glycoalkaloids.

Mycotoxins were detected in 22 samples; beauvericin was most frequent (11/22), followed by enniatins B (8/22), fumonisins B1/B2 (6/22), deoxynivalenol, ochratoxin A (5/22), and zearalenone (2/22). Only one sample would exceed the EU aflatoxin B1 limit (0.10 µg/kg) if intended for infants and young children. Multi-mycotoxin co-occurrence (2 to 5 toxins) was observed in several products. Cyanogenic glycosides were below the LOQ in all samples, while glycoalkaloids were detected in five samples (1.0 to 2.9 mg/kg), well below indicative levels (3). Powdered products demonstrated good homogeneity (RSD <5%).

The study revealed a relatively high occurrence of mycotoxins, with beauvericin and enniatins frequently detected. While most levels were below regulatory limits, the absence of clear labelling raises concerns for vulnerable populations such as infants and young children. Multi-mycotoxin contamination highlights the need for ongoing surveillance due to potential additive or synergistic toxic effects.

The study was financed by Dutch Food and Consumer Product Safety Authority (NVWA) under the project *Method development, accreditation maintenance and surveys mycotoxins and plant toxins in food (WOT-02-001-061)*.

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3.77 P77 - Balancing Matrix, Mesh and Metrology: Cross-Platform Performance of Naturally Contaminated Mycotoxin Reference Materials

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Naturally contaminated reference materials are indispensable for realistic method validation and interlaboratory comparability in mycotoxin analysis [1]. Unlike spiked materials, they reflect authentic matrix–analyte interactions, extraction behavior, and distribution heterogeneity. However, their production requires careful control of particle size, homogeneity, stability, and statistically robust value assignment.

This study presents a structured production and characterization concept for naturally contaminated maize and complex feed material. The novelty lies in integrating controlled particle size engineering with a metrologically transparent value-assignment strategy and a long-term isochronous stability design. The reference materials were milled to US mesh 50, achieving high homogeneity while maintaining extractability. Beyond homogeneity optimization, a central element of this work is the implementation of a three-year isochronous stability study. Triplicate units were stored at +4°C and –20°C and analyzed simultaneously after defined intervals up to 36 months. This design minimizes analytical variability and allows direct assessment of long-term integrity and fitness-for-purpose under realistic storage conditions.

Characterization was performed via an interlaboratory study including 13 laboratories and 15 independent datasets applying LC–MS/MS, HRMS, and complementary techniques. Statistical evaluation followed ISO 13528 principles with formal outlier testing, assessment of distribution behavior, and the use of robust estimators where required [2]. A structured decision framework, considering number of laboratories, methodological diversity, combined uncertainty, and metrological traceability, was applied to assign certified, indicative, or informative values in line with ISO 17034 concepts. Certified values were obtained for major regulated mycotoxins, demonstrating strong interlaboratory harmonization. Importantly, characterization was also successful for selected non-regulated or emerging analytes, including acetylated deoxynivalenol derivatives, fumonisin B3, and moniliformin, illustrating the potential of naturally contaminated materials to support extended multi-analyte scope.

In contrast, analytes such as enniatins, nivalenol, and deoxynivalenol-3-glucoside revealed significant between-laboratory variability and insufficient robustness for value

assignment, highlighting ongoing challenges in harmonization and calibration strategies for these compounds. Limited cross-platform assessment confirmed general applicability beyond chromatographic techniques, though with analyte-dependent constraints. Overall, this work demonstrates that the combination of controlled particle size optimization, long-term isochronous stability assessment, and a transparent, statistics-based value-assignment approach enables the production of naturally contaminated multi-analyte materials suitable for both regulated and emerging mycotoxins.

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3.78 P78 - Validation of an LC–MS/MS Multi-Mycotoxin Method in Plant-Based Meat Alternatives: Brand-Level Assessment Across Soy, Wheat, and Seitan Matrices

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Plant-derived meat alternatives are increasingly consumed as sustainable protein sources, yet their complex, processed matrices pose challenges for chemical safety assessment, including mycotoxin monitoring. Robust, matrix-appropriate validation of analytical methods is therefore essential to ensure reliable exposure assessments and regulatory compliance.

We present validation data for an established LC–MS/MS multi-mycotoxin method applied to three major categories of plant-based meat replacements: soy-, wheat-, and seitan-based products. In line with recent EU validation guidance emphasizing the assessment of matrix effects across individual samples rather than relying on technical replicates, the study design used seven distinct commercial brands per category. This approach captures inter-sample variability arising from differing ingredients, processing aids, and formulations typical of these products.

The validation covers repeatability, intermediate precision, limits of quantification (LOQ), recoveries of the extraction and matrix effects. Calibration was matrix-matched within each category to mitigate ionization bias, and performance was evaluated for a representative panel of regulated and emerging mycotoxins commonly monitored in cereals and cereal-derived foods.

LOQs were comparable to those previously obtained in raw grains, indicating no material loss of sensitivity despite the higher fat, protein, and additive content often present in plant-based meat analogues. Repeatability and intermediate precision met the commonly applied criterion of RSD<20% for almost all analytes across all three categories. Matrix effects were generally slight, while recoveries of the extraction were somewhat lower than in raw grains, probably due to the substantial water content (up to 50%) in many products, which causes a slight dilution of the extract.

3.79 P79 - Rising Risks in Europe: Key Findings from the 2025 Mycotoxin Survey

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Introduction

The dsm-firmenich World Mycotoxin Survey 2025 shows that Europe is facing rising mycotoxin pressures, with several regions now classified as *extreme-risk* due to increasing levels of all six major mycotoxins. These trends indicate a broader climate driven shift in contamination patterns and highlight the need for reinforced monitoring and mitigation strategies across the European feed sector.

Materials and Methods

In 2025, a total of 25,626 samples sourced worldwide were analyzed for major mycotoxins, aflatoxins (Afla), zearalenone (ZEN), B-trichothecenes (B-Trichos), A-trichothecenes (A-Trichos), fumonisins (FUM) and ochratoxin A (OTA). Of these samples, 10,795 originated from Europe. Analyses were performed using liquid chromatography coupled to tandem mass spectrometry, high performance liquid chromatography, and enzyme-linked immunosorbent assay.

Results

The data clearly show- that Europe is facing a significant mycotoxin challenge in 2025, with many regions marked in high-risk categories. Corn-based feed materials are especially affected. Corn silage samples are almost universally contaminated, with 93% testing positive for deoxynivalenol (DON) at notably high average levels (1,636 µg/kg). Corn grain also shows widespread multi-toxin contamination, with DON detected in 72% of samples, followed by high occurrence of FUM (65% at an average of 1,293 µg/kg) and ZEN (52% occurrence at an average level of 241 µg/kg).

Across all sample groups, B-Trichothecenes (such as DON) stand out as the most prevalent mycotoxins (73% occurrence), closely followed by ZEN (54%) and FUM (48%). Median and maximum concentrations indicate not only frequent but also high-intensity contamination, increasing the risk for livestock health and feed quality.

Conclusion

Overall, the data of 2025 highlight a multi-toxin challenge with 81% of all tested samples containing more than one mycotoxin. This high co-occurrence underscores the need for robust monitoring and effective mitigation strategies to protect European feed supply chains and livestock systems.

3.80 P80 - The presence of moulds and mycotoxins in animal feed collected between 2024 and 2025

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Mycotoxin contamination remains a major concern for food and feed safety worldwide. Climate change, including rising temperatures and altered humidity levels, is significantly influencing the distribution of moulds and associated mycotoxins. It is predicted that species such as *Aspergillus flavus* and *Fusarium graminearum* will become more common in Europe in the coming decades, increasing the risk of crops contamination with aflatoxins and *Fusarium* toxins. These changes in the distribution of the fungal species in Europe are expected to modify exposure patterns, resulting in people being exposed to different mycotoxins with varying health effects. Contamination with multiple mycotoxins, including mixtures of aflatoxins, fumonisins, deoxynivalenol and zearalenone, is expected to increase in crops, thereby escalating human dietary exposure.

Certain mycotoxins are prioritised in European food safety regulations due to their toxicity and prevalence in agricultural produce. However, the European Union has not established maximum levels for total mould counts in animal feed. In the context of animal feed and fungal contamination, it is not usually the presence of mould or yeast itself that poses a significant health risk. Contamination by non-mycotoxin-producing mould species at levels of up to 1,000,000 colony-forming units (CFU)/g may potentially result in a 5–10% loss of energy value, but does not pose a significant risk to animals. Therefore, safety controls for animal feed focus primarily on the presence and concentration of specific mycotoxins. There is currently no clear correlation between viable mould counts and mycotoxin levels.

The aim of this study was to evaluate the levels of moulds and mycotoxins in animal feed collected between 2024 and 2025. Trichothecenes and zearalenone were analysed using LC-MS/MS, while ochratoxin A (OTA) and aflatoxins were determined using HPLC-FLD. All 394 analysed samples contained deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZEN), with median concentrations of 90 µg/kg, 19 µg/kg, 5 µg/kg, 11 µg/kg and 9 µg/kg, respectively. The highest concentrations were observed in pig feed in 2024, reaching 5,761 µg/kg for DON and 1,377 µg/kg for ZEN. OTA was found in 69% of the 181 samples analysed (median 0.41 µg/kg; maximum concentration 6 µg/kg). No aflatoxins were detected in the analysed samples.

Mycological examination was carried out using yeast extract glucose chloramphenicol (YGC) agar. Samples were incubated for 5-7 days at 25 ± 1°C, and results were expressed as CFU/g of sample. The moulds were identified at the genus level. In 2024, 4% of feed samples exceeded 1,000,000 CFU/g of fungi, whereas in 2025 this proportion increased to 17%. *Fusarium* spp. dominated in animal feed at 42% in 2024, followed by *Penicillium* spp. (35%). In contrast, *Aspergillus* spp. were dominant in 2025 (44%).

These findings confirm that moulds and mycotoxins remain widespread contaminants in animal feed and feed materials, underscoring the need for continuous monitoring and preventive strategies.

3.81 P81 - Zearalenone and α -zearalenol Modulate Cell Cycle Progression and Affect NRF2 Signaling in an Inflammation-Based Model of Breast Cancer Cells

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Obesity-associated chronic low-grade inflammation is a recognized promoter of breast cancer (BC) initiation and progression. Inflammatory mediators released by adipose tissue create a microenvironment that enhances proliferation, survival, and metabolic reprogramming of tumour cells. Mycotoxins with estrogenic activity, including zearalenone (ZEA) and its metabolite α -zearalenol (α -ZOL), may further modulate these processes; however, their impact under inflammatory conditions relevant to obesity-related BC remains poorly characterized.

In this study, we examined the effects of ZEA and α -ZOL in an inflammation-induced model of human breast cancer cell lines. Both compounds significantly altered cell cycle progression, indicating disruption of proliferative control under cytokine-driven stress. Additionally, our results demonstrate modulation of the NRF2 pathway, suggesting effect on mitochondrial biogenesis and cellular stress responses. These findings imply that ZEA and α -ZOL may potentiate inflammation-enhanced oncogenic mechanisms through coordinated effects on cell cycle regulation and NRF1-dependent transcriptional activity.

Our data highlight the importance of considering dietary exposure to estrogenic mycotoxins in the context of obesity-associated BC risk. The observed molecular changes offer new insights into how environmental xenoestrogens interact with inflammatory signaling to influence breast cancer cell behavior.

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3.82 P82 - The Interaction of a Blend of Non-Starch Polysaccharide Enzymes with Deoxynivalenol, and its Modified Form Deoxynivalonol-3-Glucoside, on the Growth Performance and Toxicokinetic in Broiler Chickens

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Deoxynivalenol-3-glucoside (DON-3G) is a metabolite produced when the plant infected with *Fusarium sp* conjugates DON to glucose via enzymatic action as a defense mechanism. After reaching the digestive tract of mammals, DON-3G suffer nonspecific hydrolysis by the host microbiota generating free DON and contributing to an increased mycotoxin exposure (1). In poultry, it has been suggested that DON-3G is not hydrolysed to DON (2). As poultry diets are commonly supplemented with exogenous enzymes to enhance the nutrient availability, the interaction of DON-3G with some enzymatic cocktails containing also cellulase should be considered. For instance, to avoid increased intestinal viscosity caused by non-starch polysaccharides (NSP) in broiler chickens, diets are often supplemented with NSP enzymes (or NSPases), which are composed of xylanases and glucanases and sometimes may contain cellulase. In this study, performed in broiler chickens, we assessed the interaction of DON, DON-3G, and a blend of NSPases on the growth performance, levels of DON and metabolites in excreta (Experiment 1), and in a toxicokinetic study (Experiment 2). In the Experiment 1, the birds were fed maize-based diets naturally contaminated with low (LD; 516-792 µg DON/kg diet) or moderate (MD; 2395-3020 µg DON/kg diet) DON levels. The LD and MD diets were split into two sub-batches; one sub-batch was supplemented with a blend of NSPases while the other remained non-supplemented. In experiment 2, the same diets were used in a toxicokinetic study. The MD diets impaired the growth of the broiler chickens up to 14 days of age, but no differences were observed in the older birds. Although NSPase increased excreta levels of DON-3-sulfate (DON-3S) in 28-day-old birds, no effects were observed in growth performance. The NSPase blend improved growth performance, even though it affected the shape of the toxicokinetic curve in broilers fed MD diets and increased the circulating DON levels in broiler chickens fed the LD diet. As far as we know, the present data show for the first time that NSPases interfere with DON toxicokinetics in broiler chickens.

3.83 P83 - Common mycotoxins in dry dog and cat feed

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For maintaining animal health, nutrition is of central importance and high-quality feed is a prerequisite. One group of possible natural contaminants is mycotoxins. While there are numerous studies investigating the mycotoxin content of feed for farm animals, only limited studies are available for contamination in pet feed. Therefore, within two diploma theses work we investigated the occurrence of common mycotoxins in dry dog and cat feed.

Samples (25 each) were collected in retail stores in the Eastern part of Austria with a special focus on feed listing cereals as ingredient. The samples were stored appropriately as specified by the manufacturer and grinded. A representative sample (5 g) was weight-in, extracted with the fourfold amount of acetonitrile-water-acetic acid (79:20:1, v/v/v) and diluted according to Sulyok *et al.* (2006) prior to analysis (1). General liquid-chromatographic mass spectrometric methods in positive and negative electrospray ionization mode were applied for analysis using a Sciex QTrap 6500+. External calibration curves were used for quantitative determination, and the results were evaluated using descriptive statistical methods.

As expected, deoxynivalenol and zearalenone were detected in almost all samples, but at rather low concentrations. Furthermore, the presence of fumonisins was confirmed in more than 50% of the samples. Concerning the emerging mycotoxins, one cat feed sample showed considerable contaminations (> 100 µg/kg) with alternariol and alternariol monomethyl ether.

Concluding, the contamination of pet feed with contaminants and in particular mycotoxins should not be neglected, and monitoring processes should be in place to ensure pet animal health.

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3.84 P84 - To bind or to degrade? Fate of deoxynivalenol and ochratoxin A during soy fermentation

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Mycotoxins are toxic compounds produced by filamentous fungi that can contaminate crops such as soy or wheat (1). With the growing popularity of soy-based fermented foods in Europe, understanding the behaviour of mycotoxins during fermentation is a food safety necessity. Ochratoxin A (OTA) and deoxynivalenol (DON) are two structurally distinct mycotoxins that may contaminate soy products. OTA is known to bind to microbial cell-wall polysaccharides (2), while DON shows limited binding (3). This study investigated the binding or metabolization of OTA and DON in fermented soy-based medium (SBM). Liquid SBM was inoculated with the lactic acid bacterium *Lactiplantibacillus plantarum* or the yeasts *Zygosaccharomyces rouxii* or *Saccharomyces cerevisiae*, and solid SBM with the fungi *Aspergillus oryzae* or *Actinomucor elegans*. For the liquid SBM, the whole culture and the cell-free supernatant (containing only unbound mycotoxin), were extracted. Cultures were extracted using Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) method followed by Oasis MAX SPE (Waters), except for liquid SBM with DON and its controls, and analysed by high-resolution mass spectrometry. Significant OTA binding was observed for *L. plantarum* (68% ± 5%, $p = 1.19E-08$), *Z. rouxii* (45 % ± 4 %, $p = 1.61E-07$), and *S. cerevisiae* (34 % ± 5 %, $p = 4.17E-05$), whereas DON showed no binding. Degradation was measured in presence of fungi with for DON 62% ± 10 %, $p = 4.98E-04$ for *A. oryzae*, 47 % ± 4 %, $p = 1.97E-09$ for *A. elegans* and for OTA 27% ± 6 %, $p = 7.01E-08$ and 24 % ± 8 %, $p = 6.65E-05$, respectively. Comparison with literature data suggests that mycotoxin binding is dependent amongst other on microbial strain, matrix composition, and incubation time (4). In this study, no commonly described degradation products were detected (5–7). However, several metabolites appeared only in OTA- or DON-contaminated and fermented medium, pointing to potential other mycotoxin transformation products during fermentation. These results suggest that in fermented SBM, OTA is more effectively removed through adsorption to microbial biomass in a species-dependent manner, whereas DON is mainly eliminated via fungal degradation. However, toxicity of those degradation compounds should be further investigated. Overall, this study highlights the importance of considering both adsorption and degradation mechanisms when evaluating the fate of mycotoxins in fermented foods.

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3.85 P85 - Messy matrix, clean results: optimising sample clean-up for ochratoxin A and deoxynivalenol detection in fermented soy

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Recently, an increasing number of soy-based fermented foods have appeared on the European market as alternatives to animal products or as novel flavours, making it essential to ensure their food safety. While the Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) method is widely appreciated for its simplicity and efficiency, highly complex matrices can still compromise analyte detection (1). Fermentation increases matrix complexity through microbial metabolites that may interfere with analyte signals. Although soymeal-based matrices can be extracted using QuEChERS and analysed by mass spectrometry (MS), fatty acids in the acetonitrile extract affect extraction and detection. Therefore, the aim of this study was to identify the most effective clean-up procedure to increase the signal-to-noise for two mycotoxins with distinct physicochemical properties: ochratoxin A (OTA) and deoxynivalenol (DON). In this study, a soymeal-based medium inoculated and fermented with *Aspergillus oryzae* (fungus used to produce koji, as a base for soy sauce or miso) was extracted using the QuEChERS method and subjected to a variety of clean-up strategies. The evaluated approaches included hexane liquid-liquid partitioning, solid-phase extraction (SPE) using Oasis MAX (Waters), Oasis MCX (Waters), Oasis HLB (Waters), and C18 (Waters) sorbents, and QuEChUP with and without Enhanced Matrix Removal-Lipid (Agilent Technologies) SPE clean-up (1–3). All extracts were analysed in untargeted mode by high resolution MS (HRMS). Among the methods tested, Oasis MAX SPE provided the most efficient overall clean-up, improving analyte-matrix separation, reducing background noise, and enhancing detection, with signal increases of 980 % ± 57 for OTA and 373 % ± 19 for DON compared to direct QuEChERS extract. The main difference was that OTA was effectively retained on the Oasis MAX SPE cartridge due to its anionic properties, whereas DON eluted with the matrix fraction. In conclusion, Oasis MAX SPE helps to reduce matrix interferences and improve detection sensitivity, making it a promising approach for routine monitoring of OTA and DON in fermented soy-based foods. This work highlights the analytical challenges posed by fungal fermentation and co-extracted lipids, and provides a practical solution for enhancing analytical reliability in complex food matrices. Beyond fermented soymeal-based medium, this approach could be applied to other fermented food products and adapted to monitor additional contaminants.

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3.86 P86 - Occurrence of T-2 and HT-2 mycotoxins in different spring barley varieties and concentration changes during the malting process

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Barley (*Hordeum vulgare L.*) is one of the most important cereal crops, widely used for animal feed, malt production, and the food industry [1]. Ensuring the quality and safety of barley raw material is essential, particularly due to potential contamination with mycotoxins that pose risks to both human and animal health [2,3]. This study aimed to determine whether higher concentrations of T-2 and HT-2 mycotoxins occur in malting or feed spring barley varieties, and to evaluate how these concentrations change during the malting process.

A two-year field experiment was conducted using one malting and one feed spring barley variety. Each variety was grown in four field replicates, and laboratory analyses were performed in duplicate. For malting, barley samples with the highest initial T-2 and HT-2 contamination levels were selected. The malting process included three day steeping in distilled water at 14°C, followed by three days of germination at 45% grain moisture and 14°C. Kilning began at 55°C for 12 h, with the temperature increased by 5°C every 1.5 h up to 80°C, followed by 4 h at 80°C. T-2 and HT-2 concentrations were determined using high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS).

Over the two growing seasons, the highest concentrations of T-2 and HT-2 toxins were detected under typical Lithuanian climatic conditions. In contrast, wetter conditions during vegetation or prior to harvest were associated with increased concentrations of other mycotoxins, while T-2 and HT-2 levels were lower. In the year with the highest T-2 and HT-2 contamination, feed barley showed higher toxin concentrations than malting barley; however, due to high variability among replicates, the differences were not statistically significant. During malting, T-2 and HT-2 concentrations decreased nearly threefold after steeping. However, during germination, toxin levels increased more than twofold compared to the end of steeping. After kilning, concentrations were lower than at the beginning of malting but still exceeded the threshold of 50 µg/kg. No significant differences in mycotoxin concentrations were observed between malting and feed barley varieties.

The results demonstrate that although technological processing steps may partially reduce mycotoxin levels, malting alone cannot guarantee concentrations below European Union regulatory limits if the raw material is initially contaminated. Residual toxins persist, and their removal from contaminated grain is practically impossible. Therefore, the initial level of contamination in raw barley is a critical determinant of final product safety and quality.

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3.87 P87 - Development and validation of a LC-MS/MS multi-method for the determination of mycotoxins in insect-based novel food

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In response to the global challenge of food sustainability, it has emerged that insects might be considered a promising solution to human and animal nutrition, because of their excellent protein contents. By comparison to the traditional supply chains (meat, poultry, and fish), entomoculture stands out for its high cost-effective use of resources, such as a significantly smaller water areas and volumes; such versatility proves useful for the integration into both rural and urban contexts.

Furthermore, the insect's capability to grow on various organic substrates allows to transform sub-products of the food industry into valuable resources, concretely promoting the circular economy principles.

Between 2021 and the beginning of 2023, the EU implemented a series of regulatory acts (Reg. (EU) 2017/2470) (2) legalising the consumption of six products based on four main insects' species, i.e. *Acheta domesticus*, *Alphitobius diaperinus*, *Tenebrio molitor* and *Locusta migratoria*, officially stating their entrance into the human food market as novel foods. As recommended by EFSA, official control must closely monitor the potential health risks of these products. Although the available data are still limited, edible insects can serve as vectors for microbial pathogens and environmental contaminants (heavy metals, dioxins, PCBs and mycotoxins) due to several possible contamination patterns linked to production environmental condition or insect's diet, up to the use of chemical and pharmacological products during breeding phases (3).

In consequence of increasing control needs and in view of possible new national monitoring plans, the IZSUM, as official control laboratory, has developed a multimycotoxin method that allows the simultaneous analysis of 9 mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, zearalenone, deoxynivalenol, T-2 and HT-2 toxins) through LC-MS/MS. The method has been validated according to regulation (EU) 2023/2782 (4) showing analytical performance compliant with the requirements. Validation data show recovery values, by isotope dilution, between 92% and 117% at LOQ level (aflatoxins B1, B2, G1, G2, 1 µg/kg, ochratoxin A 0.5 µg/kg, zearalenone 10 µg/kg, deoxynivalenol 100 µg/kg, T-2 and HT-2 toxins 5 µg/kg) and LOQx10 (except for aflatoxins and deoxynivalenol tested at LOQx5) with RSDr between 2 and 14%. After validation, 31 samples were purchased from both Italian producers and online market and analyzed. All samples were mycotoxins free (<LOQs) except for two, contaminated with deoxynivalenol (around 100 µg/kg) but still below the limit of Regulation (200 µg/kg).

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3.88 P88 - Development and Evaluation of a Lateral Flow Immunoassay for Tenuazonic acid

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Food safety is a central societal concern, strictly regulated by food law. Nevertheless, approximately 25 % of foods exceed legally prescribed limits¹, underscoring the need for continuous monitoring. Conventional detection methods, particularly chromatographic techniques such as LC-MS/MS, are sensitive but not suitable for on-site use in food processing. To avoid storage delays and delayed results in mycotoxin contamination, on-site analyses are essential. Immunoassays, widely used in clinical diagnostics, offer a means for on-site analysis and a practical alternative when specific antibodies are available.

In this study, we report the development of a lateral flow immunoassay (LFIA) against TeA (tenuazonic acid), a toxic metabolite produced by the genus *Alternaria* and frequently contaminating fruits, vegetables, and derived products.

For the development of the indirect LFIA, gold nanoparticle–antibody conjugates were prepared based on a passive adsorption strategy. To characterize the stability of the conjugates, UV–Vis spectra were recorded, and intensity as well as peak shifts were analyzed. Cross-reactivity and matrix effects were also investigated. Additionally, sensitivity studies were conducted to determine the visual limit of detection (vLOD) and the calculated limit of detection (cLOD), based on image analysis with ImageJ. Furthermore, preliminary reference material was measured.

A prerequisite for this detection system is the preparation of gold nanoparticle–antibody conjugates in which the antibody is passively adsorbed onto the citrate-coated surface of the gold nanoparticles in a carbonate buffer (pH 10.6). The conjugation requires an optimal antibody concentration of 25 µg/mL. Initial results show clear, reproducible signals that correlate with TeA concentration. The LFIA has a run time of 15 minutes and a visual LOD (vLOD) of 25 ng/mL, as well as a calculated instrumental LOD of 1.75 ng/mL.

Future work will extend the detection system to additional mycotoxins, including alternariol (AOH) and patulin. Moreover, the development of a multiplex LFIA is planned to enable simultaneous detection of different mycotoxins in a single analysis.

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3.89 P89 - *Fusarium* infection and mycotoxin production in *Cannabis sativa*: Implications for crop safety and consumer health

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Cannabis sativa is a highly domesticated plant cultivated worldwide for medicinal, recreational, and industrial applications, including fiber, grain, and cannabinoid production(1). The economically valuable floral tissues are increasingly threatened by fungal contamination, particularly by *Fusarium* species, which are associated with diseases such as *Fusarium* head blight (FHB), and their ability to produce toxic secondary metabolites, mycotoxins(2), (3). These toxins, including trichothecenes, zearalenone, and fumonisins, may compromise crop quality and pose potential health risks to consumers, especially immunocompromised individuals(3). Despite these concerns, current regulatory frameworks primarily target aflatoxins and ochratoxin A, leaving many *Fusarium* mycotoxins insufficiently monitored(1). To address the gap between agricultural contamination and consumer exposure, this study investigated mycotoxin occurrence in cannabis plants. An LC–MS/MS method was optimized for the simultaneous detection and quantification of hundreds of metabolites, including mycotoxin-related compounds. Forty *C. sativa* plants were cultivated and inoculated with seven *Fusarium* isolates derived from hemp seeds, followed by genomic DNA extraction and sequencing for pathogen identification. Infectivity and production of mycotoxins and other secondary metabolites were evaluated. Over 70% of detected metabolites were *Fusarium*-derived, and T-2, HT-2, and deoxynivalenol were identified in several samples. Furthermore, over fifty hemp samples obtained from Austrian markets and online sources will be analyzed to determine their mycotoxin profiles using LC-MS/MS. These findings characterize the mycotoxin profile of hemp and highlight that understanding *Fusarium* contamination is critical for improving disease management, risk assessment, and product safety within the rapidly expanding cannabis industry.

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3.90 P90 - Occurrence of T-2, HT-2 and Their Glucosides in Apple-Based Beverages

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Apple juice is the most-consumed juice in Germany and apple products such as purees or smoothies are especially popular products for infants and children [1]. Apples are widely cultivated in Germany and there are a wide range of apple varieties grown.

T-2- and HT-2-toxins are secondary metabolites mainly produced by the genus *Fusarium*. Main producers are *Fusarium sporotrichoides*, *F. poea* and *F. langsethiae*. They are primarily associated with cereals such as oats or wheat, where maximum levels have been established (e.g. 100 µg/kg in oats, 20µg/kg in other cereals) [2]. Based on toxicity data, the European Food Safety Authority (EFSA) has established a group tolerable daily intake (TDI) of 0.02 µg/kg body weight for T-2, HT-2 and their modified forms [3]. These modified forms may occur as sugar conjugates (e.g. glucosides) which are formed in the phase-II metabolism of plants. They are of particular interest because the parent toxins may be released again during digestion [4]. The consumption of apple products contaminated with T-2/HT-2 may contribute to dietary exposure especially for toddlers and infants [3].

During a routine screening for mycotoxins, T-2 and HT-2 were unexpectedly detected in apple juice. *Fusarium* toxins are not routinely monitored in fruit-based beverages, as mycotoxin control mainly focuses on patulin [5]. Therefore, this clearly indicates a previously unconsidered contamination pathway. The aim of the study was to investigate the occurrence and concentration of T-2, HT-2 and their glucosides in apple juice, apple-pear juice and apple cider from the German market and to evaluate whether apple-based beverages could contribute to dietary exposure.

For sample preparation a QuEChERS (quick, easy, cheap, effective, rugged and safe) based extraction and clean-up was applied. The quantification was conducted using liquid chromatography tandem mass spectrometry (LC-MS/MS), enabling the determination of T-2, HT-2, T-2- α -glucoside, T-2- β -glucoside, HT-2- α -Glucoside and HT-2- β -glucoside. By optimizing the liquid chromatographic gradient, separation of the α - and β -anomers was successfully achieved, which allowed a qualitative and quantitative analysis of these individually. A total of 65 samples were analysed, revealing summed concentrations up to 28 µg/L. The results provide new insights into a previously overlooked contamination pathway and suggest that apple-based beverages should be considered in future monitoring and dietary exposure assessments of T-2 and HT-2-toxins.

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3.91 P91 - *Metarhizium* spp. encode an ochratoxin cluster and a high efficiency ochratoxin-degrading amidohydrolase

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Ochratoxins (OTs) are worldwide regulated secondary metabolites due to their multitude of deleterious effects on humans and animals alike. Several *Aspergillus* and *Penicillium* spp. synthesize OTs from a six-gene biosynthetic gene cluster (BGC) that contains a halogenase, *OtaD*, to produce the highly toxic final product OTA. Here we report that three species of the distant phylogenetic taxon *Metarhizium* contain an expressed OT BGC but lack an *otaD* gene, which indicates the non-halogenated precursor OTB should be detected in these stains. Unexpectedly, no OT BGC products were found in *Metarhizium* spp. Instead, *Metarhizium* metabolized both OTA and OTB to their non-toxic degradation products OT α and OT β respectively. This activity of *M. brunneum* was attributed to an intracellular hydrolase MbAmh1, which was tracked by bioactivity-guided proteomic analysis combined with in vitro reaction. Protein structure-based assay identified a similar working model for OTA degrading enzymes. Identification of some active sites supported the important role of metal iron for this enzymatic reaction. Recombinant MbAmh1 (5 $\mu\text{g}/\text{mL}$) completely degraded 1 $\mu\text{g}/\text{mL}$ OTA within 3 min, demonstrating a strong degrading ability towards OTA. Additionally, MbAmh1 showed considerable temperature adaptability ranging from 30 to 70 $^{\circ}\text{C}$ and acidic pH stability ranging from 4.0 to 7.0. Therefore, it might have great potential in detoxifying OTA for industrial applications. These findings reveal different patterns of OT synthesis in fungi and provide new strategies to identify mycotoxin degrading enzymes.

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3.92 P92 - A glutathione-S-transferase from *Fusarium graminearum* inactivating trichothecenes by epoxide opening: a role in self-protection or fungus-fungus interaction?

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It has been proposed that the *Fusarium* resistance gene *Fhb7* from wheat grass (*Thinopyrum*) has been acquired from an *Epichloë* fungal endophyte by horizontal gene transfer [1]. This gene encodes a GST capable of opening the epoxide in trichothecene toxins. We tested several fungal homologs and found a few which are more stable than *Fhb7* protein when expressed in *E. coli* and have better kinetic properties with deoxynivalenol as substrate. *Fusarium graminearum* also has a similar gene (currently with an incorrectly annotated gene model), which confers high level resistance to DON and other trichothecenes when expressed in baker's yeast. Likewise, a GST from the non-trichothecene producing *Trichoderma reesei* confers resistance to the potent antifungal compounds trichothecin (obtained from *Trichothecium roseum*) and against trichodermin (from *Trichoderma brevicompactum*), and the deacetylated products trichothecolone and trichodermol, respectively. Potentially, these enzymes could play a role in self-resistance in the toxin producers or in fungus-fungus antagonism. We disrupted the *F. graminearum* GST gene, both in wild-type and in a *tri5 tri101* double mutant. Unaltered high-level resistance to externally added DON was observed, indicating that other mechanistically different self-resistance mechanisms are involved, or that additional GSTs in the *Fusarium* genome may have an overlapping function. A possible role of this GST in resistance against foreign trichothecenes (trichodermin, trichothecin, T-2 toxin) and the interaction with the respective producing fungi is under investigation

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3.93 P93 - ThermELUTE Light – Evaluation of an accelerated sample preparation for quantification of aflatoxins and ochratoxin A by SMART cartridges

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Aflatoxins and ochratoxin A are the most stringent regulated mycotoxins. Many commodities are susceptible to infection by fungi and mycotoxin could be found in high concentrations within this food or feed stuff. Aflatoxins and Ochratoxin A could be found in many matrices and could lead to trading rejection due to exceeding regulatory limits.

The regulatory demands in trading and food safety in food production requires constant testing of mycotoxins - high mycotoxin prevalence and exceedance of regulatory limits in batches lead to delays in production and high costs in product recall action from market to ensure food safety standards. To ensure food safety and reduce the risk of mycotoxin related product recall new technologies were developed and allow faster food testing. Compliance with food safety standards in regards to sample analysis and sample cleanup, an accelerated sample cleanup was evaluated, combined with a thermal elution of mycotoxins from the AflaCLEAN SMART or OtaCLEAN SMART cleanup column facilitating a “ready-to-inject” sample, without drawback for chromatographical separation, identification or sensitivity. The process could be performed offline (manually) or by semi- or full automated sample processing. Applicable in analytical laboratories and process-accompanying in food and feed industry in Food QC laboratories.

The ThermELUTE Light processed eluate after immune affinity cleanup could be provided for direct injection and allows monitoring far below baby food level in accelerated sample processing pathway. More than 50% of solvents per sample could be saved and at least 60% of time per sample- saving resources but still provide higher analytical sensitivity by adjusting injection volume in a regular liquid chromatography approach (HPLC-FLD). This cleanup was evaluated with corn, peanut, hazelnut for aflatoxins, dairy product like milk for aflatoxin M1 or coffee, cocoa, spices, corn and liquorice for ochratoxin A (exemplary data will be presented). Good results, faster sample preparation time and matrix compatibility facilitates faster monitoring to comply food safety standards and higher reliability to materials passing food production line.

Consistency in results and chromatographical clearance by antibody-specific concentration of analytes and deprivation of matrix interferences results in better chromatography and higher analytical sensitivity could be achieved in less time/sample, providing a tool for higher sample throughput and “in-time” simultaneous food safety check in food production chains.

We present consistency data, linearity of recovery, matrix compliance and recovery compliance to official standards by an easy-processing of samples using the SMART cartridge processing steps as a workflow model to streamline mycotoxin sample cleanup. This allows not only general saving in costs and time per sample (by reducing solvents and waste) but facilitates a mycotoxin monitoring throughout food production lines to ensure highest food quality during food processing prior to release even below baby food levels for aflatoxins and ochratoxin A.

4 Index of Authors

Acevedo Ramos, Fernando	126	Bouša, Daniel	97
Adam, Gerhard.....	22, 225, 229	Bouša, Martin	97
Agyarkó, Edward	153	Bover-Cid, S.	193
Al-Ayoubi, Carine	81	Brányik, Tomáš	167
Ali, Omeralfaroug.....	153	Breitenmoser, Lena.....	29
Alías-Segura, Sergio.....	88, 126	Bucoñ, Klaudia	99
Aliyi, Ibsa	171	Bühler, Susanne	79
Alrahal, Rafat.....	205	Bürstmayr, Hermann.....	201
Álvarez, Micaela	41	Büschl, Christoph	22
Amakhobe, Truphosa	108	Call, Florian	59
Ambichl, Verena	215	Camardo Leggieri, Marco	42
Andrade, Claudia de	111	Campmajó, Guillem	24
Anelli, Pamela	44	Caporali, Angela	222
Annunziato, Alessandro	44	Caprai, Elisabetta	180
Arczweska-Wlosek, A.	214	Carella, Daria	44, 45
Arnich, Nathalie	52, 165	Carl, Peter	100, 121, 128, 224
Atallah, Elham	90	Castellari, M.....	193
Atanasova, Vessela.....	37	Castex, Mathieu	81
Audebert, Marc	156	Castro, Óscar.....	196
Bahlmann, Arnold	27, 119	Cervellieri, Salvatore	19
Bai, Feng-Yan.....	47	Chochois, Vincent	33
Balková, Darina	42	Ciriaci, Martina	222
Balmas, Virgilio.....	44	Cirlini, Martina	130
Balogh, Krisztián.....	153	Cobigo, Louis-Marie	52
Bandara, Nandika	21, 144, 145	Codina, Raquel	196
Barenstrauch, Margot	52	Correia Costa, Jean	35
Bartucz, Cintia.....	189	Costa, Jean C.C.P.....	161
Battilani, Paola	42	Coton, Emmanuel	46, 194
Běláková, Sylvie.....	91	Coton, Monika	46, 165, 194
Benešová, Karolina.....	91	Couvert, Olivier	165
Berendika, Marija.....	28, 158	Cozzi, Giuseppe.....	44
Bergen, Janice	92	Cramer, Benedikt	105, 152, 226
Berger, Beatrice	94, 149, 174	Crequer, Ewen	46
Berger, Marion	78	Crudo, Francesco ..	59, 76, 102, 104, 178
Berntssen, Marc HG	160	Csenki, Zsolt.....	189
Berthiller, Franz.....	22, 229	Cunha, Sara C.....	39, 105, 182
Béthune, Kevin	33	Dabisch-Ruthe, Mareike.....	106
Biarnés, Xevi.....	196	Dall'Asta, Chiara... 24, 68, 104, 110, 130,	164
Bibi, Rita	222	Đalović, Ivica	138
Bier, Frank.....	224	Dam, Ruud van.....	150
Bilińska, Gabriela.....	169	Damjanović, Marko	170
Birse, N.....	207	Dänicke, Sven.....	79
Blockland, Marco.....	56	Dantigny, Philippe	194
Boba, Aleksandra	114	Dawud, Sheger	171
Bock, Illés	189	De Girolamo, Annalisa	19, 48, 108
Bogić, Ivana	138	De Saeger, Sarah	83, 108, 171
Borsos, Eszter.....	95	De Santis, Barbara.....	131, 168
Boško, Rastislav.....	91, 97	Debegnach, Francesca	131, 168
Böttcher, Christoph	149		

Del Favero, Giorgia	57, 63, 92, 102, 104, 178
Del Vecchio, Lorenzo	130
Dellafiora, Luca	66, 68, 104, 110, 160, 164
Đermanović, Branislava	170
DeRosa, Maria C.	19
Di Marco Pisciotano, Ilaria	75
Díez Arias, David	111
Dole, Léna	33
Dolezalova, Tereza	112, 191
Domańska, Anna	114, 142
Dorin, Carmen	28
Drul, Dainna	144, 145
Dubajić, Jovana	116
Duché, Henri-Olivier	52
Durand, Noel	33
Dzuman, Zbynek	191, 207
Ebel, Frank	106
Eder, Christine	102
Eerden, E. van	214
El-Hafi, Jasmin	117
El-Khatib, Ahmed H.	119
Elsner, Svenja	121
Erena Ortega, Carmen	123
Evgeniou, Michail	50
Faijes, Magdalena	196
Faino, Luigi	45
Faria, Miguel A.	39, 182
Farkaš, Hunor	116, 125
Feliho, Mirabelle	108
Fellinger, Christian	92
Fernandes, José O.	105, 182
Ferrús, Delfin	196
Fontana, Angélique	33
Forleo, Tiziana	19, 108
Frahm, Jana	79
Fratzke, Franziska	100, 128
Frederico Rodrigues Loureiro Bracarense, Ana Paula	72, 81, 198
Freitag, Stephan	201
Fugita Muranaka, Thaís	198
Fuhrmann, Samuel	29
Furmeg, Sanja	158
Galaverna, Gianni	130
Gambacorta, Lucia	48
Gámiz-Gracia, Laura	39
García de la Camacha Selgas, Nuria	126
Garnham, Christopher P.	81
Garofalo, Marion	70
Gascón-Corella, Silvia	61
Gatto, Angela	44
Geelen, Nick	205
Gianelli, Gianluigi	130
Gierth, Lukas	163
Gilbert, Matthew K.	84
Gil-Serna, Jéssica	41, 84, 88, 123, 126
Giovinazzo, Robert	33
Giraud, Tatiana	46
Göthel, Markus	100, 128, 224
Gozzi, Marco	130
Grajewski, Jan	211
Greer, B.	207
Grgic, Dino	63, 95, 102
Grieco, Martina Enza	131, 168
Grümpel-Schlüter, Angelika	79
Grzetic Martens, Josipa	205
Guàrdia, M. D.	193
Guéraud, Françoise	70
Gufler, Judith	133, 136
Gützkow, Kim Lara	134, 193
Habauzit, Denis	52, 216, 218
Hadush, Kokeb T.	108
Hager, Sonja	63, 133, 136
Haidukowski, Miriam	44
Han, Da-Yong	47
Hart, Leon	200
Hartinger, Doris	210
Henry, Jean-Michel	184
Hernández-Mesa, Maykel	39
Hetzschold, Nick	95
Hiddink, Mees	150
Holinska, Kateřina	185
Hornecker, Victoria	195
Horst, Dominique van der	205
Huber, Roman	140
Hübner, Florian	172
Humpf, Hans-Ulrich	102, 105, 152, 172, 226
Husøy, Trine	75
Hutzler, Christoph	27, 119
Hymery, Nolwenn	194
Jäckel, Udo	78
Jadhav, Akash Kalyanrao	130
Jakovčević, Zdenka	116
Janavičienė, Sigita	220
Janić Hajnal, Elizabet	138, 146
Jany, Jean-Luc	46
Jarošová Kolouchová, Irena	167
Jedziniak, P.	207
Jeßberger, Nadja	200
Jobst, Maximillian	63
Jourdan, Christophe	33
Jung, Christian	27
Kalinina, Svetlana	172
Kalischko, N.	207

Kalogiouri, Natasa	28	Lohmann, Mark.....	163
Kanonier, Pia Sophie	140	Lorenz, Nicole	27
Karner, T.....	207	Lucassen, Jesper	205
Karpiesiuk, Krzysztof	86	Lucchetti, Dario.....	131
Kenjeric, Lidija	229	Ma, Junning	54
Kerbaje, Boutros.....	97, 184	Magnaldi, Ilaria	164
Kersten, Susanne.....	79	Malachova, Alexandra	225
Kittler, Sophie.....	200	Maldonado, Maria Luisa	83
Klar, Stefanie.....	78	Malir, Frantisek.....	185
Kluess, Jeannette	79	Marchal, Camille	165
Knappstein, Karin	134	Marín, Sonia.....	31, 35, 161
Kochiieru, Yuliia.....	220	Marko, Doris	59, 63, 76, 92, 95, 102, 104, 133, 136, 178
Kochneva, Yelizaveta.....	142, 169	Marske, Lennart.....	78
Kodikara, Chamali	21, 144, 145	Martín, B.....	193
Kokić, Bojana.....	170	Martiník, Jan	91, 167
Kořacin, Aleksandra	74	Masiello, Mario.....	44
Kollberg, Susanne.....	128	Matjašec, Nuša	57
Kongcheep, Benjawan	125	Maul, Ronald.....	117, 134, 193
Konthur, Zoltán	100, 128, 224	Mauro, Simona	131, 168
Korz, Sven.....	174	Max, Maryse	65
Kos, Jovana.....	146, 170, 207	McKeon, Hannah P.....	75
Kosicki, Robert	99, 169, 188, 211	Meiners, Torsten.....	149
Kövesi, Benjámín	153	Melguizo, Clara	84
Kowalska, Karolina	74, 148	Mengellers, Marcel J.B.....	75
Kowarschick, Stefanie	140	Mézes, Miklós	153
Kozera, Wojciech.....	86	Michlmayr, Herbert	229
Kozieł-Leszczyńska, Marta Justyna ...	148	Mierziak, Justyna	114, 142, 169
Krais, Annette.....	29	Migheli, Quirico	44
Krause, Sophie.....	149	Miljanić, Jelena	146, 170
Krska, Rudolf	176, 201, 209, 225, 229	Miljić, Milorad.....	138
Krstović, Saša	170	Minkoumba Sonfack, Gaetan	180
Kruijt, Alwin.....	150	Mirey, Gladys.....	70, 156
Kudumija, Nina.....	158	Mohammed, Abdi	26, 171
Kühn, Jakob.....	56	Moinard, Magalie.....	37
Kühnhenrich, Nina.....	105, 152	Molino, Francisco.....	31
Kulcsár, Szabina.....	153	Montaser, Mehrsima	172
Kulišová, Markéta.....	167	Monzón-Atienza, Luis.....	66, 68, 160
Kulma, Anna	114, 142	Moore, Geromy G.	84
Kumpum, Watinee	125	Moretti, Antonio	24, 44, 45, 48, 108
Kwiatkowska-Giżyńska, Justyna.....	154	Muñoz, Katherine	174
Lalaurie, Margaux.....	70	Nalpadan, Avon Augustin.....	86
Lamp, Julika.....	134	Narvaéz, Alfonso	39
Lattanzio, V.	207	Naud, Nathalie	70
Le Moël, Nina	156	Nazareth, Tiago.....	31, 35
Léon, Eva	196	Neuhoff, Judith	78
Lepczyński, Adam	86	Noldin, Anika	79
Lešić, Tina.....	158	Nøstbakken, Ole Jakob..	66, 68, 110, 160
Lie, Kai Kristoffer	66, 68, 110, 160	Novak, Barbara	176, 210
Lim-Trinh, Andy.....	136	Nyathi, Lunghani	108
Lindh, Christian	29	Obermoser, Florian.....	215
Lippi, Yannick	70, 81	Ochieng, Kevin	108
Lippolis, Vincenzo.....	19, 108	Okott, Sheila	108
Llorens, Enric.....	31, 35, 161		

Orsini, Serenella	222	Prats, Annabel.....	196
Oster, Michael.....	86	Prim, Montserrat	161
Ostry, Vladimir	185	Přinosil, Aleš	97
Oswald, Isabelle P.	61, 70, 76, 81, 90	Prizio, Ilaria	180
Oufensou, Safa	44	Prusova, Nela	112, 191
Pacetti, Adela	185	Psota, Vratislav	97
Pareek, Chandra Shekhar	86	Puel, Olivier	46, 76
Partsch, Vanessa	178	Puel, Sylvie.....	70, 81
Pascari, Xenia	56	Puzyński, Bartosz	114
Patiño, Belén.....	41, 84, 88, 123, 126	Quinteros, G.	193
Patriarca, Andrea	59, 83	Radović, Radmila.....	146
Paulino Leite Gomes, Ana Laura .	72, 198	Raj, Jog.....	116, 125
Pavicich, María Agustina	83, 171	Ramos, Antonio J.	31, 35, 161
Pawtowski, Audrey.....	46	Ramos, Helena	182
Payros, Delphine	70	Ratzer, Noah	76, 102
Pecorelli, Ivan.....	207, 222	Réant, Charlotte.....	194
Pedroni, Lorenzo ..	66, 68, 104, 110, 160, 164	Rehagel, Christina	195, 200
Peloso, Mariantonietta.....	180	Reinert, Jessica	78
Penary, Marie.....	70, 81	Renaud, Justin B.....	81
Perak Junaković, Eleonora.....	158	Rennhofer, Patrick	201
Perduh, Bojana.....	146	Reyer, Henry	86
Pereira, Cheila	105, 182	Riahi, Insaf	196
Pernica, Marek	91, 184	Richard-Forget, Florence.....	37
Perugino, Florinda	104	Righetti, Laura.....	75
Petitpierre, Anouk.....	29	Rives, Clémence	70, 81
Petronijevic, Filip.....	22	Rodriguez Ferraz, Camila	72
Pfannebecker, Jens.....	106	Rodriguez, Alicia	84
Pfordt, Annette	48	Ropars, Jeanne.....	46
Phuengkasem, Anawach.....	125	Rousseau-Bacquié, Elodie	70
Piastowska-Ciesielska, Agnieszka		Rubira Gerez, Juliana	72
Wanda	74, 148, 213	Russo, Katia	131
Picicci, Irene	24	Sachse, Benjamin	27
Pickova, Darina.....	185	Saltzman, Janine	79
Pierre, Fabrice	70	Samanidou, Viktoria F.	28
Pierzchała, Mariusz	86	Sanahuja, Raquel	196
Pilarska, Gabriela.....	186	Santos, R.R.	214
Pinson-Gadais, Laetitia	37	Šarić, Ljubiša	146
Pinto, Eugénia	39	Sawuła, Agnieszka.....	169
Pinton, Philippe	70, 81	Sayuri Kono, Isabelli.....	198
Planas, Antoni	196	Schale, Daniela.....	200
Platzer, Alexander	50	Schamann, Alexandra	225
Pleadin, J.	207	Schatzmayr, Dian	176
Pletzer, Malena	224	Schepens, Marloes A. A.....	75
Plewa-Tutaj, Kinga.....	188	Schneider, Rudolf J.....	100, 128, 224
Plötz, Madeleine	200	Schöberl, Astrid.....	201
Poirier, Elisabeth	194	Schorr-Galindo, Sabine	33
Ponsuksili, Siriluck	86	Schröder, Christian	57, 92
Poór, Miklós	189	Schütte, Tanja	94
Porqueddu, Giampiera	44	Sdogati, Stefano.....	222
Pötter, Dierk-Christoph	78	Seeger, Bettina	200
Pour Nikfardjam, Martin	226	Selimagić, Amina.....	178
Praskova, Barbora	112	Serra, X.	193
		Sirot, Véronique.....	52

Skrzydlewski, Paweł	99, 203, 211	Urbanek, Kinga Anna	213
Ślaska, Brygida	86	Usleber, Ewald	200
Šmíd, František.....	97	Valente, Nina	229
Søfteland, Liv Ingeborg.....	66, 160	Van Cauwenberge, Camille	83
Sokolović, Marijana	28, 158	Van Pamel, Els.....	216, 218
Soler, Laura	76, 81, 90, 156	Van Poucke, Christof.....	216, 218
Somma, Stefania	44	Varga, Elisabeth	95, 215
Sopel, Marta.....	75, 150, 205	Varsalona, Maria.....	19
Soszczyńska, Ewelina.....	188, 211	Vasiljević, Marko.....	116, 125
Sousa Monteiro, Carolina	39, 105	Veerkamp, Jannik.....	105, 226
Souza, Marielen de.....	72	Venier, Sita	216, 218
Sprong, Corinne.....	75	Venslovas, Elimantas	220
Stawiarz, Radosław	169	Verdini, Emanuela.....	222
Steinborn, Silke	78	Vergin, Chantal	100, 128, 224
Steiner, D.	207	Verri Junior, Waldiceu Aparecido.....	72
Storz, Maximilian Andreas.....	140	Verstockt, Maarten	83
Stranska, Milena.....	112, 191	Vettorazzi, Ariane	61
Straubinger, Reinhard K.	106	Vignard, Julien	70, 156
Strub, Caroline	33	Villani, Alessandra.....	44, 45
Sudwischer, Patrick	79	Villot, Clothilde	81
Sulyok, Michael .176, 201, 207, 209, 225		Visioli, Giovanna	130
Suman, M.	207	Visoka, Yllka	225
Sumarah, Mark W.	81	Völsch, Annalisa M.....	105, 226
Sun, Lu.....	54	Vukić, Milan	138
Susca, Antonia.....	24, 44, 45	Vukosi, Edwin.....	108
Svoboda, Zdeněk	91	Vulić, Ana.....	158
Sweany, Rebecca R.....	84	Waesoh, Nazmi	63
Swiatkiewicz, S.....	214	Walczak-Szeffer, Anna	213
Szabó, András.....	153	Wang, Gang	54, 228
Szabó, István	189	Wang, Xue-Wei	47
Tadessa, Tullu.....	171	Weigel, Stefan.....	27, 56, 65, 119
Tai, Bowen.....	54	Weinstabl, Magdalena	92
Taschl, Ines.....	176, 210	Wesonga, John.....	117
Tesouro Rodriguez, Anna	111	Westerhout, Joost.....	75
Thebault, Anne.....	165	Wiesenberger, Gerlinde	22, 229
Thill, Sam.....	59	Wimmers, Klaus	86
Tittlemier, Sheryl A.....	21, 144, 145, 207	Wittich, Georg.....	195
Toman, Jakub	185	Wolf, Anja	78
Tomanić, Dragana	146	Wongviriyakit, Siwalaj.....	125
Tóth, Gergő	189	Wu, Wenqing	228
Touya, Aurélie	37	Wuppermann, Frederik N.	230
Trakooljul, Nares	86	Xing, Fuguo	54, 228
Trentzsch, Markus.....	65	Yeo, Charles	108
Tripon, Roberta	28	Yousefvand, Amin	111
Tulcan, Camelia	28	Zadravec, Manuela	158
Twaruschek, Krisztian.....	229	Zalewski, Iwan.....	142
Twarużek, Magdalena 99, 154, 169, 186, 188, 203, 211		Zándoki, Erika	153
Ulrich, Sebastian	106	Zanim Michelazzo, Paola.....	198
		Zinsstag, Lucienne.....	29

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Society for Mycotoxin Research (Gesellschaft für Mykotoxinforschung e.V.)

The Society for Mycotoxin Research (Gesellschaft für Mykotoxinforschung e.V.) was founded in Munich, Germany, in 1997. The aim of this registered, non-profit association is to promote research on all scientific fields of mycotoxinology. One of the core ideas of the Society was to create bridges between the different scientific disciplines working on the field of toxigenic fungi and mycotoxins. The Society presently has around 150 members from 20 countries, organises the annual Mycotoxin Workshop and it publishes the journal *Mycotoxin Research*. Further information on the Society and membership can be found at www.mycotoxin.de.