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ROLE OF MUSHROOM SUBSTRATE IN THE TRANSFER OF FUNGICIDE RESIDUES TO *Agaricus bisporus*

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ABSTRACT

White button mushroom (*Agaricus bisporus*) is a significant component of the human diet due to its nutritional value, which includes digestible proteins, dietary fiber, phenolic compounds, and B-group vitamins. In recent years, interest in organic agricultural products has increased, accompanied by growing demands for food quality and safety. Organically cultivated edible fungi, produced without the use of plant protection products, are generally expected to be safe and health-promoting. However, despite the application of organic production methods, the presence of pesticide residues in mushrooms remains possible, thus requiring comprehensive analysis.

This study aimed to evaluate the presence of plant protection products residues in mushroom fruiting bodies cultivated using substrates from conventional and organic sources. A total of 158 mushroom samples and 128 substrate samples were analysed using QuEChERS-based methods to assess whether the growing substrate facilitates the pesticide transfer to mushrooms. The results revealed significant differences between organic and conventional mushrooms. However, residues in mushrooms were detected even without the direct application of pesticides during cultivation, indicating that chemical substances can be transferred from the substrate to fruiting bodies. The presence of residues in mushrooms labeled as organic suggests that substrate contamination is an underestimated pathway for pesticide transfer. The findings emphasize the need for more precise regulation of substrate materials employed in mushroom cultivation, particularly within organic systems, to ensure food safety and compliance with residue limits. Monitoring mushroom substrates is crucial for developing sustainable and safe agricultural practices.

Keywords: edible fungi, food safety, organic cultivation, plant protection products, pesticide transfer, QuEChERS-based methods

INTRODUCTION

The white button mushroom (*Agaricus bisporus*) is one of the most commonly consumed mushrooms in the world. Poland is the leader in mushroom production in Europe, with annual production exceeding 330,000 tons, 70% of which is exported [Siwulski et al. 2022, Smoleński 2022]. The production of organic mushrooms, like other vegetable crops, constitutes a relatively small percentage of the total output. However, this market demonstrates a consistent growth [Weber and Skorbiansky 2023]. These mushrooms

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are distinguished not only by their taste and availability, but also by their high nutritional value. They are a source of easily digestible protein, fibre, vitamins (mainly from the B group, but also D, E and K), microelements (Se, K, Cu) and bioactive compounds such as ergothioneine and polysaccharides with antioxidant, immunostimulating, antidiabetic and anticancer properties [Golak-Siwulska et al. 2018, Mattila et al. 2001, Muszyńska et al. 2017, Sidor 2019].

One of the most significant factors affecting human health and well-being is proper nutrition, including the quality of the food products consumed. The increasing demand for organic food has resulted in greater attention to food safety and the absence of chemical contaminants. However, the intensification of agricultural practices to achieve high yields of qualified plants has increased the need for chemical crop protection, thereby resulting in the growing importance of assessing food of plant origin for fungicide residues [Bursić et al. 2021, European Food Safety Authority 2019, Gomiero 2018].

Organic mushroom cultivation involves the exclusion of plant protection products from the production process. Nevertheless, the risk of their presence can be attributed to the use of raw materials in the production of mushroom substrate. There is a significant knowledge gap concerning the migration of pesticide residues from the substrate to the *A. bisporus* fruiting bodies, despite the recognised possibility of such penetration [European Food Safety Authority 2016, 2019, Goglio et al. 2024]. The substrate for mushroom cultivation is composed of high-quality cereal straw (mainly wheat and triticale), poultry manure, gypsum, and water [Tello Martin et al. 2022, Wang et al. 2023]. Straw is derived from crops, which are frequently cultivated using conventional methods. Despite the continuous use of fungicides in intensive wheat production, research on their potential impact on mushroom cultivation remains limited. Fungicide applications during wheat cultivation can result in residues in grain or straw. Current knowledge on how fungicides used in wheat cultivation may affect mushroom production is still incomplete. However, there is a risk that residues of growth regulators, fungicides, and other pesticides may end up in the substrate, even though the mushroom cultivation process itself is carried out without their use [Chalaux et al. 1993, Tello Martin et al. 2022, McGee 2018]. Consequently, the fruiting bodies may become contaminated via the substrate. In the context of organic mushroom production, raw materials must meet specific criteria and possess the necessary certificates that attest to their suitability for organic farming, as outlined in the general regulations for organic products standardized by Commission Regulation (EC) no 149/2008.

Accordingly, it is imperative that straw and chicken droppings must be sourced from certified farms, free from pesticide residues and cereal growth regulators. Gypsum used to reduce substrate pH, must be uncontaminated and produced without involving power plant processes for purifying exhaust gases from sulfur. An annual audit is conducted by the relevant certification body to verify compliance with the established standards for the production of organic substrate for mushroom cultivation. The responsibility for regulating the reliability of inspection bodies is held by the Inspection of Trade Quality of Agricultural and Food Products, operating under the authority of the Ministry of Agriculture and Rural Development [Regulation (EU) 2018/848].

In recent years, the presence of pesticide residue has attracted particular attention from food safety authorities. In this regard, the European Union has introduced a series of regulations and legislation, including Commission Regulation (EC) no 149/2008 and the Food and Nutrition Safety Act, which obliges all Member States to adhere to comprehensive food control measures. These measures focus on the presence of contaminants, including pesticides, in food products. The European Union has set maximum permissible levels for these compounds, also known as MRLs (maximum residue levels), which are specific to food products of plant origin, including mushrooms [Commission Regulation (EC) 149/2008, Stachniuk and Fornal 2016].

The objective of the present study was to assess the presence of pesticide residues in both the substrate and the fruiting bodies of *A. bisporus* from organic and conventional crops. The study particularly emphasized the presence of cereal growth regulators, such as chlormequat chloride and mepiquat chloride, as well as fungicides used in cereal cultivation. Additionally, the investigation involved the analysis of pesticides applied in mushroom cultivation.

MATERIAL AND METHODS

Analyzed samples

Samples of mushroom substrate and *A. bisporus* fruiting bodies were obtained from production facilities located in various regions of the country during the period 2021–2024. The samples analysed in this study were obtained from crops cultivated using conventional and organic methods (Table 1). The weight of each sample was 1 kg.

The samples were submitted to the Food Safety Laboratory (FSL) within 24 hours of collection and were assessed as undamaged and suitable for pesticide residue testing. The samples were immediately cooled to –18 °C and ground using dry ice. All procedures related to sample preparation and analysis were consistent with generally accepted procedures and guidelines for official laboratories in the EU [Document Nº SANTE/11312/2021v2 2021].

Analytical methods

To assess pesticide residues in samples tested, the following research methods were employed:

QuEChERS multiresidue method. This method is based on the EN 15662:2018 standard [European Committee for Standardization 2018]. In general, the method consists of using QuEChERS extraction with acetonitrile, followed by purification of the extracts using dispersive solid-phase extraction (d-SPE), and then subjecting the extracts to analysis using gas and liquid chromatography coupled with tandem mass spectrometry. The detailed procedure is as follows: 10 g of a soil sample was weighed into a 50 mL Falcon tube, and 10 mL of acetonitrile was added. The mixture was then vortexed for approximately 3 min. Then, a mixture of salt and extraction buffer (4 g of anhydrous magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate dihydrate, and 0.5 g of sodium hydrogen citrate) was added and shaken for 3 min. The sample was centrifuged using a laboratory centrifuge for 5 min (7200 rpm, room temperature), and 1 mL of the supernatant was transferred to an Eppendorf centrifuge tube containing the purification mixture (150 mg of magnesium sulfate and 25 mg of PSA) and shaken for approximately 1 min. The sample was centrifuged using an Eppendorf MiniSpin centrifuge for 1 min. (8500 rpm, room temperature). The extracts prepared in this way were subjected to further analysis using two chromatographic techniques: gas and liquid chromatography.

For gas chromatography, 1 mL of the extract was transferred to an autosampler cup. Then, 50 µL of internal standard, 100 µL of acetonitrile, and 30 µL of

Table 1. Origin of mushroom substrates and fruiting body samples

Sample	Production category	Number of samples	Sampling voivodeship
Mushroom substrates	organic production	41	łódzkie (substrate producer)
		19	łódzkie (mushroom producer)
		2	wielkopolskie (mushroom producer)
	conventional production	23	łódzkie (substrate producer)
		40	łódzkie (mushroom producer)
		3	mazowieckie (mushroom producer)
Mushroom fruiting bodies	organic production	41	łódzkie (mushrooms producer)
		2	wielkopolskie (mushroom producer)
		15	wielkopolskie (market)
	conventional production	68	łódzkie (mushroom producer)
		2	wielkopolskie (mushroom producer)
		6	mazowieckie (mushroom producer)
		14	łódzkie (market)
		10	mazowieckie (market)

Table 2. Scope of substances analyzed in analytical methods

Method	Substances analyzed
QuEChERS Multiresidue (499 substances)	2-phenylphenol, abamectin, acephate, acetamiprid, acetochlor, aclonifen, acrinathrin, alachlor, aldicarb, aldicarb sulfone, aldicarb sulfoxide, aldrin, allethrin, ametoctradin, ametryn, amidosulfuron, aminocarb, amisulbrom, anthraquinone, atrazine, azaconazole, azadirachtin, azinphos-ethyl, azinphos-methyl, aziprotryne, azoxystrobin, beflubutamid, benalaxyl, bendiocarb, benfluralin, benfuracarb, benthiavalicarb isopropyl, benzovindiflupyr, bifenazate, bifenazate diazene, bifenox, bifenthrin, biphenyl, bitertanol, bixafen, boscalid, bromacil, bromfenvinphos, bromocyclen, bromophos, bromophos-ethyl, bromopropylate, bromuconazole, BTS 44595, BTS 44596, bupirimate, buprofezin, butachlor, butafenacil, butylate, cadusafos, captafol, captan, carbaryl, carbendazim, carbetamide, carbofuran, carbofuran-3-hydroxy, carbofuran-3-keto, carboxin, carfentrazone-ethyl, chinomethionat, chlorantraniliprole, chlorbenside, chlorbufam, chlordane,-cis, chlordane,-oxy, chlordane,-trans, chlorfenapyr, chlorfenson, chlorfenvinphos, chloridazon, chlormephos, chlorobenzilate, chloropropylate, chlorothalonil, chlorotoluron, chlorpropham, chlorpyrifos, chlorpyrifos-methyl, chlorsulfuron, chlorthal-dimethyl, chlorthiophos, chlothion, chromafenozide, clodinafop-propargyl, clofentezine, clomazone, clothianidin, coumaphos, crimidine, cyanazine, cyanophenphos, cyanophos, cyantraniliprole, cyazofamid, cycloate, cycloxydim, cyflufenamid, cyflumetofen, cyfluthrin, cymiazol, cymoxanil, cypermethrin, cyprazine, cyproconazole, cyprodinil, DDD-o,p', DDD-p,p', DDE-o,p', DDE-p,p', DDM, DDT-o,p', DDT-p,p', DEET, deltamethrin, demeton-S, demeton-S-methyl, demeton-S-methyl sulphone, demeton-S-methyl sulfoxide, desmedipham, desmetryn, dialifos, diazinon, dichlobenil, dichlofenthion, dichlofluanid, dichloroaniline 3,5-, dichlorobenzamide 2,6-, dichlorobenzophenone-p,p, dichlorvos, diclobutrazol, dicloran, dicofol, dicrotophos, dieldrin, diethofencarb, difenoconazole, diflubenzuron, diflufenican, dimethachlor, dimethenamid, dimethoate, dimethomorph, dimoxystrobin, diniconazole, dinitramine, dinobuton, dinoseb, dinotefuran, dioxabenzofos, dioxacarb, dioxathion, diphenylamine, disulfoton, disulfoton sulfon, disulfoton sulfoxide, ditalimfos, diuron, DMF, DMPF, DMST, dodemorph, edifenphos, emamectin B1a, emamectin B1b, endosulfan alpha, endosulfan beta, endosulfan sulphate, endrin, endrin keton, EPN, epoxiconazole, esfenvalerate, etaconazole, ethalfluralin, ethametsulfuron-methyl, ethiofencarb, ethion, ethirimol, ethofumesate, ethofumesate-2-keto, ethoprophos, ethoxyquin, ethylan, etofenprox, etoxazole, etrimfos, famoxadone, fenamidone, fenamiphos, fenamiphos sulfoxide, Fenamiphos sulphone, fenarimol, fenazaquin, fenbuconazole, fenchlorphos, fenfuram, fenhexamid, fenitrothion, fenobucarb, fenoxaprop-P-ethyl, fenoxycarb, fenpropathrin, fenpropidin, fenpropimorph, fenpyrazamine, fenpyroximate, fensulfothion, fensulfothion oxon, fensulfothion oxon sulphone, fensulfothion sulphone, fenthion, fenthion oxon, fenthion oxon sulphone, fenthion sulfoxide, fenthion sulphone, fenvalerate, fipronil, fipronil desulfinyl, fipronil sulfon, flazasulfuron, flonicamid, florasulam, fluchloralin, flucythrinate, fludioxonil, fluensulfone, flufenacet, flufenoxuron, flumetralin, flumioxazin, fluopicolide, fluopyram, fluorodifen, fluotrimazole, fluoxastrobin, flupyradifurone, fluquinconazole, flurochloridone, flurprimidol, flurtamone, flusilazole, flutianil, flutolanil, flutriafol, fluxapyroxad, folpet, fonofos, foramsulfuron, formetanate, formothion, fosthiazate, fuberidazole, furalaxyl, furathiocarb, gamma-cyhalothrin, halfenprox, halofenozide, heptachlor, heptachlor cis-epoxid isomer B, heptachlor trans-epoxid isomer A, heptenophos, hexachlorobenzene, hexachlorocyclohexane HCH alpha, hexachlorocyclohexane HCH beta, hexaconazole, hexaflumuron, hexythiazox, imazalil, imazapic, imidacloprid, indoxacarb, iodofenphos, iodosulfuron methyl, ipconazole, iprobenfos, iprodione, iprovalicarb, isocarbophos, isofenphos, isofenphos-methyl, isofetamid, isoprocarb, isoprothiolane, isoproturon, isopyrazam, isoxaben, isoxaflutole, isoxathion, kresoxim-methyl, lambda-cyhalothrin, lenacil, lindane, linuron, lufenuron, malaoxon, malathion, mandestrobin, mandipropamid, mecarbam, mepanipyrim, mepronil, metaflumizone, metalaxyl, metamitron, metazachlor, metconazole, methacrifos, methamidophos, methidathion, methiocarb, methiocarb sulphone, methiocarb sulphoxide, methomyl, methoprotryne, methoxychlor, methoxyfenozide, metobromuron, metolachlor, metolachlor-S, metosulam, metoxuron, metrafenone, metribuzin, metsulfuron-methyl, mevinphos, molinate, monocrotophos, monuron, myclobutanil, napropamide, nicosulfuron, nitenpyram, nitralin, nitrapyrin, nitrofen, nitrothal isopropyl, novaluron, nuarimol, omethoate, oxadiazon, oxadixyl, oxamyl, oxycarboxin, oxyfluorfen, paclobutrazol, paraoxon-methyl, parathion, parathion-methyl, penconazole, pencycuron, pendimethalin, penflufen, pentachloroaniline, penthiopyrad, permethrin, pethoxamid, phenmedipham, phenthoate, phorate, phorate sulfone, phorate sulfoxide, phosalone, phosmet, phosmet oxon, phosphamidon, phoxim, phthalimide, picolinafen, picoxystrobin, pinoxaden, piperonyl butoxide, piperophos, pirimicarb, pirimicarb desmethyl, pirimiphos-ethyl, pirimiphos-methyl, prochloraz, procymidone, profenofos, profluralin, prometon, prometryn, propachlor, propamocarb, propaquizafop, propargite, propazine, propetamphos, propham, propiconazole, propoxur, propoxycarbazone, propyzamide, proquinazid, prosulfocarb, prosulfuron, prothioconazole destio, prothiofos, pymetrozine, pyraclostrobin, pyrazophos, pyrethrins, pyridaben, pyridafol, pyridalyl, pyridaphenthion, pyrifenox, pyrimethanil, pyriofenone, pyriproxyfen, pyroquilon, pyroxsulam, quinalphos, quinclorac, quinoclamine, quinoxyfen, quintozene, quizalofop-ethyl, resmethrin, rimsulfuron, rotenone, saflufenacil, silafluofen, silthiofam, simazine, spinetoram c42, spinetoram c43, spinosyn a, spinosyn d, spirodiclofen, spiromesifen, spirotetramat, spirotetramat enol, spirotetramat enol-glucoside, spirotetramat ketohydroxy, spirotetramat monohydroxy, spiroxamine, sulfometuron methyl, sulfosulfuron, sulfotep, sulfoxaflor, tau-fluvalinate, tebuconazole, tebufenozide, tebufenpyrad, tecnazene, teflubenzuron, tefluthrin, tepraloxydim, terbacil, terbufos, terbufos oxon, terbufos sulphone, terbufos sulphoxide, terbuthylazine, terbutryn, tetrachlorvinphos, tetraconazole, tetradifon, tetrahydrophthalimide, tetramethrin, tetrasul, thiabendazole, thiacloprid, thiamethoxam, thiencarbazone-methyl, thifensulfuron-methyl, thiobencarb, thiodicarb, thiometon, thiophanate-methyl, tolclofos-methyl, tolfenpyrad, tolylfluanid, topramezone, tralkoxydim, triadimefon, triadimenol, tri-allate, triazophos, trichlorfon, tricyclazole, tridemorph, trifloxystrobin, triflumizole, triflumuron, trifluralin, triflusulfuron, triticonazole, tritosulfuron, vinclozolin, zoxamide
QuPPe-PO method	chlormequat, chlorates, cyromazine, fosetyl-al, mepiquat, perchlorates, phosphonic acid

the protector working solution (AP-MIX) were added. Extracts were analyzed using highly selective gas chromatography coupled with tandem mass spectrometry (GC-MS/MS). An Agilent Technologies 7890A gas chromatograph equipped with a 7000 Triple Quad GC/MS mass detector was used for the analyses. Compound separation was performed on a DB-5MS capillary column (30.0 m × 250 µm × 0.25 µm). Identification and quantification were performed using the Multiple Reaction Monitoring technique.

For liquid chromatography, 200 µL of the extract was transferred to an Eppendorf tube containing 700 µL of solvent A. Then, 50 µL of internal standard and 50 µL of acetonitrile were added. The whole mixture was filtered into an autosampler cup. The analysis was performed using highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). An Agilent Technologies 1200 Series liquid chromatograph equipped with a 6410 Triple Quad LC/MS mass detector was used. Compound separation was performed on an Agilent Eclipse Plus, C18, 2.1 × 100 mm, 1.8 µm column. Two solvents: A – water and 5 mM ammonium formate and 0.01% v/v formic acid, and B – water: acetonitrile (5:95) and 5 mM ammonium formate and 0.01% v/v formic acid were used. Measurements were made using an ESI ion source in positive polarity using the Dynamic Multi Reaction Monitoring ion scanning mode. The scope of the analyzed substances within the multi-residue method for the GC-MS/MS and LC-MS/MS techniques is described in Table 2. All the substances analyzed by the multi-residue method were detected at the 0.01 or 0.005 mg kg^{-1} limit of quantification (LOQ).

QuPPe-PO single residue method. The method consists of several variants, as described in guidelines published by EU Reference Laboratories [Anastassiades et al. 2023]. A method is described for the residue analysis of highly polar, non-QuEChERS-amenable pesticides. Residues were extracted from the test portion after adjusting the water content and acidifying with 1% formic acid in methanol. The mixture was centrifuged, filtered, and directly analyzed using liquid chromatography coupled with tandem mass spectrometry:

– Agilent Technologies 1260 Series liquid chromatograph equipped with a 6460 Triple Quad LC/MS mass detector. Compound separation was performed on a Zorbax Hilic Plus, 2.1 × 100 mm, 1.8 µm column. Two solvents: A – 20 mM ammonium formate with 0.36% (v/v) formic acid in water, and B – acetonitrile were used. Measurements were performed using an ESI ion source in negative polarization using the Multiple Reaction Monitoring ion scanning mode. The method was used for the determination of chlormequat chloride, mepiquat chloride, and cyromazine;

– Agilent Technologies 1290 Infinity II Series liquid chromatograph equipped with a 6470B Triple Quad LC/MS mass detector. Compound separation was performed on a Thermo Scientific Hypercarb, 2.1 × 100 mm, 5 µm column. Two solvents: A – 1% acetic acid in water, and B – 1% acetic acid in methanol and 10% water were used. Measurements were performed using an ESI ion source in negative polarization using the Multiple Reaction Monitoring ion scanning mode. The method was used for the determination of chlorates, fosetyl-Al, perchlorates, and phosphonic acid.

Quantification was performed employing isotope-labeled analogs of the target analytes as internal standards. These were added directly to the test portion at the beginning of the procedure to compensate for any factors influencing the recovery rates, such as volume deviations, analyte losses during sample preparation, and matrix effects during measurement.

Statistical methods

Statistical analysis was performed by using MS Excel 2019 with the Analysis ToolPak add-in. Pesticide residue data obtained from conventional and organic samples of substrates and mushrooms over a four-year period were analyzed. For each year, the percentage of samples containing particular active substances was calculated. Differences between residue levels in conventional and organic substrates and fruiting bodies were evaluated using Student's t-test with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Mushroom substrate from organic cultivations

The study involved the assessment of 62 samples of organic substrate, of which 21 samples (34.1%) were free from chemical residues (Fig. 1). Approximately 20% of the samples contained a single substance, 24% contained two substances, and 11% contained three

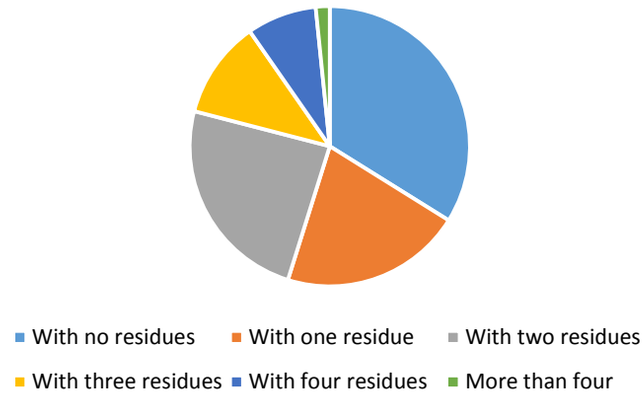

Fig. 1. Percentage of organic mushroom substrate samples with chemical residues

Table 3. Number of organic mushroom substrate samples containing chemical residues in the years 2021–2024

Year	2021	2022	2023	2024	Total
Number of samples	15	16	14	17	62
Chlormequat choride	2	4	–	1	7
Mepiquat chloride	–	–	4	–	4
Anthraquinone	–	1	2	–	3
Azoxystrobin	–	–	1	–	1
Benzovindyflupyr	–	–	1	–	1
Bromide ion	1	–	6	–	7
Chlorate	2	7	–	9	18
Epoxyconazole	–	1	–	–	1
Fluxapyroxad	2	–	–	–	2
Folpet	2	5	5	2	14
Melamine	–	–	–	7	7
Phthalimide	2	5	5	2	14
Tebuconazole	1	3	1	–	5
Tetraconazole	1	–	–	–	1
Samples without residues	9	5	3	4	21

substances. In the remaining samples, four chemical substances were detected.

Table 3 presents the chemicals detected in organic substrate samples from each year. In 2021, a total of 15 samples of mushroom substrate were analysed, of which 9 samples were free from any plant protection product residues. Two samples of substrate contained chlormequat chloride at a level of 0.007–0.012 mg kg^{-1}, while 5 samples contained other fungicides. In 2022, a total of 16 samples of organic substrate were analysed, and 5 samples did not contain any detected residues. The remaining samples contained various chemical substances. Four samples contained chlormequat chloride at 0.006–0.011 mg kg^{-1}, and 10 samples contained fungicides such as folpet and phthalimide (33% of samples) and tebuconazole (20%). Seven samples revealed the presence of chlorate residues. In 2023, 14 samples of organic substrate were analysed, of which three were free from any residues. Four samples contained mepiquat chloride at levels of

Table 4. Number of conventional mushroom substrate samples containing chemical residues in the years 2021–2024

Year	2021	2022	2023	2024	Total
Number of samples	11	18	22	15	66
Chlormequat choride	4	8	2	3	17
Mepiquat chloride	10	14	21	12	57
Azoxystrobin	5	7	5	8	25
Bixafen	–	–	–	2	2
Benzovindyflupyr	–	6	2	3	11
Bromide ion	–	–	9	2	11
Chlorate	1	6	11	5	23
Cypermethrin	1	1	2	1	4
Epoxyconazole	2	1	–	–	3
Fluxapyroxad	2	5	6	5	18
Folpet	3	9	15	5	32
Melamine	–	–	–	5	5
Phthalimide	2	9	15	5	31
Propiconazole	1	1	–	–	2
Pyraclostrobin	–	1	2	–	3
Tebuconazole	10	15	13	6	44
Tetraconazole	1	2	3	1	7
Triadimenol	3	–	–	–	3

0.005–0.052 mg kg^{-1}, as well as chemical products used in cereal protection (including bromide ion, phthalimide, and folpet). Seven samples contained residues of fungicides, mainly folpet, phthalimide, and bromide ion (54.5% of samples), as well as tebuconazole (27%). In 2024, 17 samples of organic substrate were analyzed, and of which 4 did not contain any residues. In one sample, residues of chlormequat chloride were detected at a level of 0.005 mg kg^{-1}, while in the remaining samples melamine and chlorate were identified.

The most frequently detected chemical substances in the tested organic mushroom substrate samples are presented in Figure 2. The samples were mainly contaminated with chlorates (29%), folpet and phthalimide (23%), and more than 11% contained bromide ion, melamine, and chlormequat chloride.

The frequency of detection of plant protection products residues and other substances in the substrate samples varied between years, indicating potential fluctuations in their utilisation or differences in persistence in raw materials. From 2021 to 2024, the percentage of residue-free substrate samples decreased, while the profiles of detected substances changed. A decrease in chlormequat chloride detection was observed in 2023, whilst detections of other pesticides and chlorates increased. Furthermore, over 20% of the samples were contaminated with chlorates, folpet, and phthalimide, while approximately 10% contained bromide ion and melamine.

Previous research has demonstrated that melamine residues are a prevalent occurrence in plant-based foods. Reports suggest that these may be from the metabolism of the triazine insecticide cyromazine or the triazine fungicide anilazine, or may originate from cyanamide fertilizer. The detection of melamine and its degradation products appears to be associated with the use of fertilisers containing cyanamide [Lütjens et al. 2023]. Nevertheless, the application of pesticides is prohibited in organic farming. Residues of these compounds, as well as others such as mepiquat chloride or chlormequat chloride, have been detected in mushroom substrates as a result of the use of fungicides and growth regulators in the protection of cereals [Chalaux

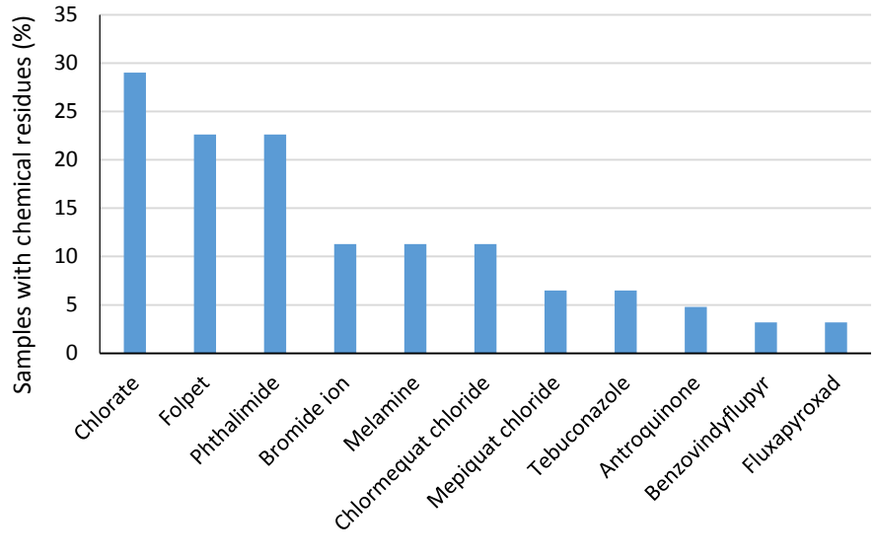

Fig. 2. Chemical residues detected in organic mushroom substrate samples

et al. 1993]. The detection of bromide ions in mushroom substrate derived from straw may be attributed to the natural bioaccumulation of bromine from the soil by wheat or other cereal crops, rather than to external contamination. Several studies have confirmed that plants can absorb bromide from the soil and water through natural physiological processes [Shtangeeva et al. 2019, Yamada 1968]. However, the application of bromine-containing pesticides in industry and agriculture resulted in an increase of bromide levels in soil. The process of bromine accumulation by plants is also influenced by the physicochemical properties of the soil [Shtangeeva 2017].

Mushroom substrate from conventional cultivations

A total of 66 samples of conventional mushroom substrate were examined. Only one sample was found to be free from chemical substances. The analysis revealed that in more than 45% of the samples, four or more of chemical substances were detected. Furthermore, 24% of the samples exhibited the presence of 4 substances, and 15% of samples contained 3 chemical substances. In the other substrate samples, 1 or 2 chemical substances were identified (Fig. 3).

Table 4 presents the number of conventional substrate samples collected each year, along with the chemicals detected. In 2021, residues of plant protection products were detected in all samples. Mepiquat chloride was detected in 10 samples, at concentrations from 0.006 to 0.029 mg/kg. Chlormequat chloride was identified in 3 samples. Furthermore, the presence of other fungicides in ten samples was detected, namely azoxystrobin, tebuconazole, folpet, phthalimide, fluxapyroxad, triadimenol, epoxiconazole, and propiconazole. In 2022, a total of 18 samples of conventional substrate were analyzed. Mepiquat chloride was detected in 14 samples, with concentrations ranging from 0.005 to 0.051 mg kg^{-1}, while chlormequat chloride was identified in eight samples. Furthermore, the following compounds were detected: tebuconazole, folpet, phthalimide, thiabendazole, azoxystrobin, benzovindiflupyr, cypermethrin, and fluxapyroxad. In 2023, mepiquat chloride was detected in 21 out of 22 samples of conventional mushroom substrate with concentrations ranging from 0.005 to 0.021 mg kg^{-1}. Two samples also contained chlormequat chloride. Folpet and phthalimide were the most frequently detected fungicides, followed by tebuconazole and chlorates. In 2024, analysis of 12 samples of conventional substrate revealed the presence of mepiquat chloride at levels of 0.008–0.014 mg kg^{-1} and chloride chlormequat at 0.006–0.009 mg kg^{-1} in 3 samples. Furthermore,

12 other chemical substances were identified in substrate samples with variable prevalence. The most frequently were: azoxystrobin, tebuconazole, folpet, phthalimide, fluxapyroxad and melamine.

Figure 4 shows the most frequently detected chemicals in the tested samples of conventional mushroom substrate. Almost 90% of the samples contained mepiquat chloride, over 60% contained tebuconazole, and more than 40% contained folpet and phthalimide. Azoxystrobin, chlorates, and chlormequat chloride were also detected in a significant proportion of samples.

Results of our study indicate that conventional mushroom substrates frequently exhibited the presence of multiple pesticide residues, with a higher probabili-

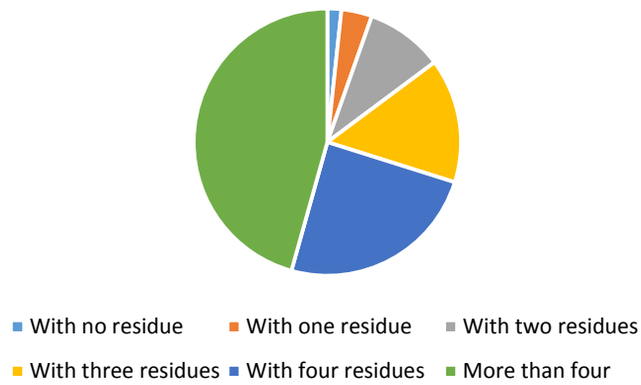

Fig. 3. Percentage of conventional mushroom substrate samples with chemical residues

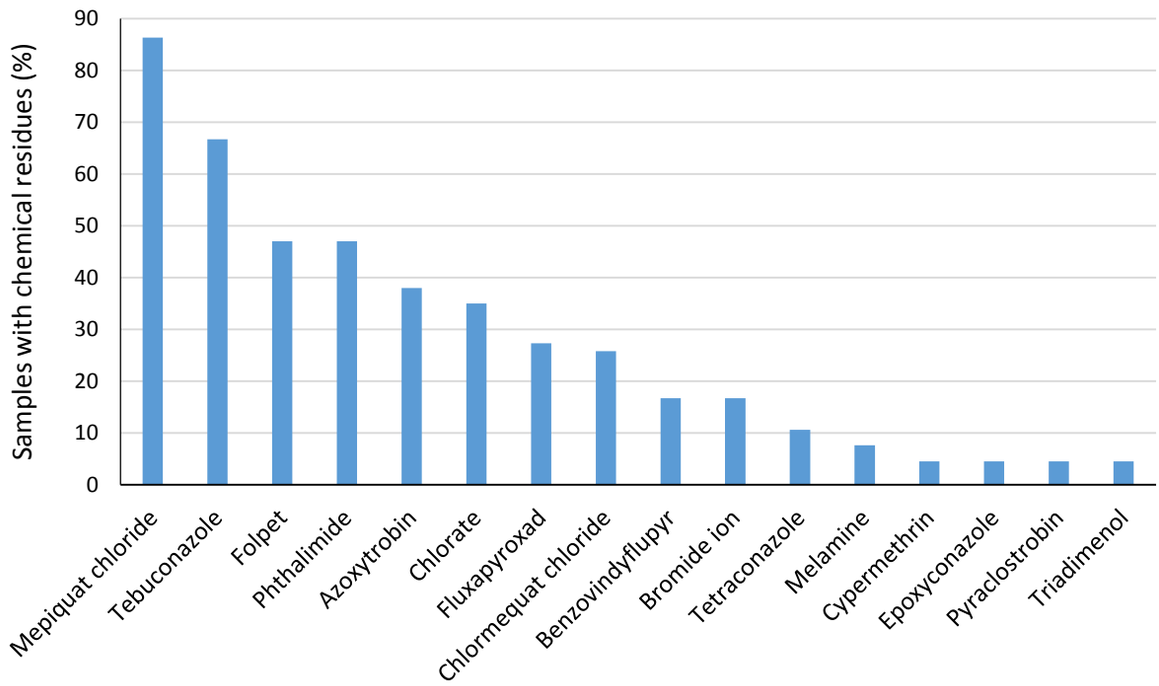

Fig. 4. Chemical residues detected in conventional mushroom substrate samples

ty of containing 4 or more residues in comparison to 3 or fewer. In some cases, analysis of individual samples revealed the presence of up to 14 different residues. Additionally, analysis of conventional substrate samples has revealed the presence of epoxiconazole and propiconazole, which have lost their EU permissions. These substances were derived from preparations previously applied for the control fungal diseases in cereal crops and were approved for use in the EU until 19/03/2020 [Commission Implementing Regulation 2018/1865; Commission Regulation 2020/749].

The occurrence of multiple pesticide residues in conventional and organic substrate samples was compared. It was stated highly significant differences between the two types of substrate. In conventional substrates, multiple residues were detected in 45%, 24%, and 15% of samples, corresponding to samples containing more than 4, 4, and 3 substances, respectively. In contrast, organic substrate samples contained multiple residues in only 2%, 8%, and 11% of samples at the same residue levels, respectively.

Statistical analysis confirmed significant differences in pesticide residues between organic and conventional substrates. Conventional samples exhibited a higher frequency of mepiquat chloride and fungicides (e.g., azoxystrobin, fluxapyroxad, tebuconazole, and tetraconazole). In contrast, chlorate, bromide ion, and melamine exhibited comparable levels across both analysed samples, while anthraquinone occurred exclusively in organic samples (5.1%). Residue-free samples were more common in the organic group (34.1%), whereas none were detected among conventional substrates. Conven-

Table 5. Number of samples with pesticide residues in organic and conventional mushroom substrates over a period of four years

Substances analyzed	Mean number of substrate samples with residues (%)	
	organic	conventional
Chlormequat chloride	11.1 ±10.8 A	27.5 ±15.9 A
Mepiquat chloride	7.1 ±14.3 B	86.0 ±8.5 A
Anthraquinone	5.1 ±5.8 A	0.0 ±0.0 A
Azoxystrobin	1.8 ±3.5 B	40.1 ±13.0 A
Bixafen	0.0 ±0.0 A	3.3 ±13.4 A
Benzovindyflupyr	3.3 ±7.1 A	3.6 ±6.7 A
Boskalid	0.0 ±0.0 A	1.4 ±2.8 A
Chlorate	27.5 ±25.0 A	31.4 ±16.8 A
Cyproconazole	0.0 ±0.0 A	1.4 ±2.8 A
Epoxynazole	1.6 ±3.1 A	5.9 ±8.6 A
Fluxapyroxad	3.3 ±6.1 B	26.6 ±6.2 A
Folpet	23.0 ±12.3 A	44.7 ±18.4 A
Fthalimide	23.0 ±12.3 A	42.4 ±21.5 A
Bromid ion	12.5 ±20.1 A	13.6 ±19.3 A
Melamine	10.3 ±20.4 A	8.3 ±17.3 A
Pyraclostrobin	0.0 ±0.0 A	3.6 ±4.7 A
Propiconazole	0.0 ±0.0 A	3.7 ±4.3 A
Tebuconazole	8.1 ±8.5 B	68.3 ±23.3 A
Tetraconazole	1.7 ±3.3 B	10.1 ±2.3 A
Triadimenol	0.0 ±0.0 A	6.8 ±13.6 A
Without residues	34.1 ±17.8 A	0.0 ± 0.0 B

Values are means of data ± standard deviation (SD); means in the same row with the same letter do not differ statistically ($P < 0.05$, Student's t-test)

tional production was found to be associated with a significantly higher prevalence of residue (Table 5).

It should be noted that the straw used in mushroom substrate production must be monitored for the chemical residues, due to the fact that fungicides are applied during the intensive wheat production in Europe and other regions of the world. Significant quantities of residues in grain or straw can result from these application, but slight information is available on possible effects of pesticides used during wheat cultivation on mushroom production.

Mushrooms from organic cultivation

An assessment of the residue of plant protection products was conducted on 58 samples of edible mushroom fruiting bodies from organic farms. The analysis revealed that approximately 40.1% of the samples were free from chemical contamination. The other samples contained mepiquat chloride (approximately 30% of samples), chlorate and melamine (15% and 12% of samples, respectively), and about 6% contained chlormequat chloride (Fig. 5).

Table 6 presents the number of fruiting body samples from organic farms from each year and the detected chemical substances. In 2021, no residues of plant protection products were detected in 61% of samples. In the remaining samples, the most common were mepiquat chloride, chlormequat chloride, and chlorates. In one sample, residues of fungicides and insecticides used in the protection of mushroom crops (metrafenone, prochloraz and cyromazine) were also found. All residue levels did not exceed the permissible limits. Table 7 presents the maximum residue limits (MRLs) for chlormequat chloride and mepiquat chloride in mushrooms, which are 0.9 mg kg^{-1} and 0.09 mg kg^{-1}, respectively, according to the European Food Safety Authority [European Food Safety Authority 2024].

In 2022, no residues were found in most fruiting body samples, while three samples contained chlorate residues at a level of 0.017–0.041 mg L^{-1}. These values were well below the permissible limit of 0.7 mg L^{-1} (Table 7). In 2023, 80% of organic samples contained mepiquat chloride residues, with an average level of 0.006 mg kg^{-1}. Moreover, no other plant protection products were detected in the mushrooms.

In 2024, two samples of organic fruiting bodies contained mepiquat chloride residues at a level of 0.006 mg kg^{-1}, and one sample contained chlormequat chloride 0.009 mg kg^{-1}. Furthermore, melamine residues were detected in all samples of fruiting bodies (0.022–0.12 mg kg^{-1}). In two samples, chlorate residues were also identified at a level of 0.015–0.35 mg kg^{-1}, not exceeding permissible levels.

The white button mushroom is cultivated on substrate derived from cereals straw. Given the extensive utilisation of chlormequat chloride in the cultivation of these crops, it is highly likely that the residues originate from mycelial uptake from the straw, rather than from direct application to mushroom cultivation. The mycelium responsible for mushroom development is characterised by extensive growth, coming into contact with a substantial mass of straw. The presence of chlormequat chloride residues in mushrooms is considered a reliable indicator of either high efficiency in the absorption of this compound or limited ability to metabolise it [Reynolds et al. 2004]. The presence

Table 6. Number of organic mushroom fruiting body samples containing chemical residues in the years 2021–2024

Year	2021	2022	2023	2024	Total
Number of samples	23	15	10	10	58
Chlormequat choride	3	–	–	1	4
Mepiquat chloride	7	–	8	2	17
Chlorate	3	3	–	2	8
Cyromazine	1	–	–	–	1
Melamine	–	–	–	10	10
Metrafenone	1	–	–	–	1
Prochloraz	1	1	–	–	1
Samples without residues	12	12	2	–	26

Table 7. Plant protection product residues detected in mushroom fruiting bodies from organic cultivation

Chemical substance	Concentration (mg/kg)	Mean	Standards range and assessment	
			MRL[1] (mg/kg)	result compliance[2]
Mepiquat chloride	0.005–0.012	0.008	0.09	compliant
Chlormequat chloride	0.006–0.012	0.008	0.9	compliant
Chlorate	0.015–0.35	0.075	0.7	compliant
Melamine	0.022–0.12	0.065	2.5	compliant
Metrafenone	0.022 ±0.011	0.022	0.5	compliant
Cyromazine	0.75 ±0.38	0.75	10.0	compliant
Prochloraz-Mn	0.038 ±0.019	0.038	3.0	compliant

[1] Maximum residues level, in accordance with Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005, as amended

[2] Decision rule for determining compliance/non-compliance according to Document Nº SANTE/12682/2019: Result is compliant, if x−U ≤ MRL; result is non-compliant, if $x-U$ > MRL

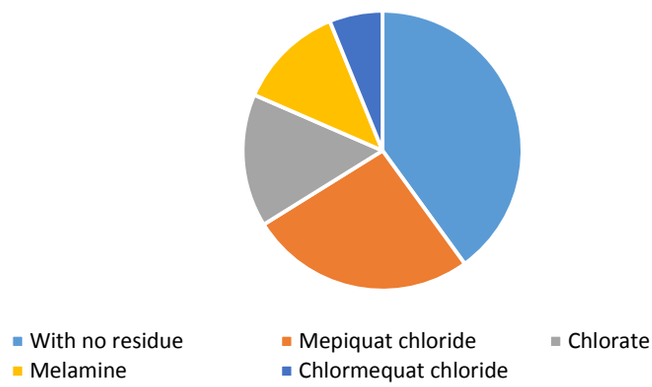

Fig. 5. Percentage of organic mushroom samples with chemical residues

of pesticides in mushrooms from organic farms have been also reported by Rembiałkowska and Badowski [2011]. Pesticides are widely applied in horticultural production worldwide, and organic farms are not isolated units within the agricultural environment. They are often located near conventional fields where pesticides are used. These chemicals can become airborne and disperse into areas where organic crops are cultivated. The efficacy of aerial pesticide applications is significantly diminished, with only approximately 25% of the chemical reaching the intended crop, while the remainder disperses into the surrounding environment. Another pathway contributing to the migration of agrochemicals is field irrigation. Water used for irrigation can transport residues via drainage canals. Water outflow from conventional crops often contains pesticide residues, which can be absorbed by plant cultivated in organic farms. Consequently, residues of chemical substances may be present in organic produce [Benbrook and Baker 2014].

Mushrooms from conventional cultivation

The study involved analyzing pesticide residues in a total of 100 mushroom samples from conventional crops. None of the samples contained any residues. The most frequently detected compound was mepiquat

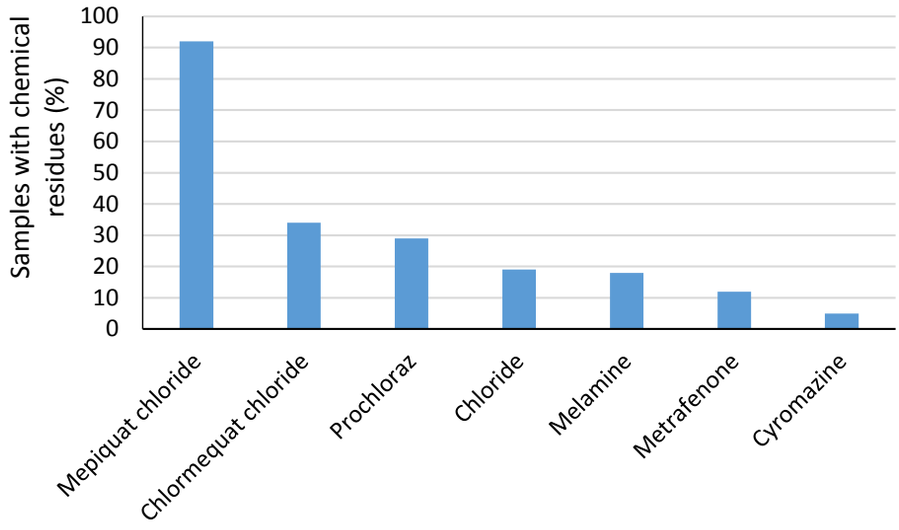

Fig. 6. Chemical residues detected in mushroom samples from conventional cultivation

chloride (92% of samples), followed by chlormequat chloride (34%) and prochloraz (29%), chlorates and melamine (20%), and metrafenone (12%), see Figure 6.

The analysis of mushroom samples collected from conventional cultivation each years is presented in Table 8. In 2021, only one mushroom sample did not contain any residues of plant protection products from straw, while the remaining samples contained chlormequat chloride and/or mepiquat chloride. Fungicide residues (prochloraz, metrafenone, or cyromazine) were detected in 13 samples, while chlorates were present in 3. All detected residues remained below the maximum residue limits (MRLs) for mushrooms (Table 9). In 2022, the residues of chlormequat chloride (0.005–0.010 mg kg^{-1}) and/or mepiquat chloride (0.005–0.050 mg kg^{-1}) were identified. Prochloraz residues were detected in eleven samples, while metrafenone and cyromazine were found in one sample. Chlorate residues were identified in three samples. In 2023, mepiquat chloride was the most prevalent detected compound. Chlorates, prochloraz-Mn and metrafenone were detected in 7, 8 and 3 samples, respectively. In 2024, mepiquat chloride was the dominant residue, while chlormequat chloride was detected in five samples. Metrafenone was present in four samples, prochloraz in one, and melamine was detected in all samples (at levels of 0.013–0.56 mg kg^{-1}).

The analysis of residues in mushrooms from conventional crops demonstrates the prevalence of chemicals in the majority of the samples. Moreover, conventional mushrooms exhibited higher prevalence of residues than those cultivated organically. A similar conclusion was drawn by Rembiałkowska and Badowski [2011], who stated that the probability of pesticide residues in organic products is reduced by a 3–4-fold compared to conventional products. The likelihood of organic raw materials containing multiple pesticides is up to 11-fold lower than for conventional materials. A large number of studies have further confirmed that the mean level of organic product contamination with pesticides is 3-fold or even 10-fold lower than the mean concentration of the same compound in conventional products. The most frequently detected pesticides in the conventional samples were tebuconazole, folpet, phthalimide and azoxystrobin, which were found in over 30% of the samples. In the organic samples, the most frequently observed compounds were folpet and phthalimide, although these were only present in 20% of the samples. Furthermore, the variation in the levels of detected fungicides was found to be significantly lower in organic samples than the conventional ones.

Table 8. Number of conventional mushroom fruiting body samples with chemical residues in the years 2021–2024

Year	2021	2022	2023	2024	Total
Number of samples	30	27	25	18	100
Chlormequat choride	10	13	6	5	34
Mepiquat chloride	26	25	24	17	92
Azoxystrobin	–	–	–	1	1
Benzovindyflupyr	–	–	–	1	1
Carbendazim	–	–	–	1	1
Chlorate	3	8	7	1	19
Cyromazine	4	1	–	–	5
Folpet	–	–	–	1	1
Melamine	–	–	–	18	18
Metrafenone	4	1	3	4	12
Phthalimide	–	–	–	1	1
Prochloraz	9	11	8	1	29
Tebuconazole	–	–	–	1	1
Tiametoxam	–	–	–	1	1
2-phenylphenol	–	–	–	1	1

Table 9. Levels of detected residues of chemical substances in conventional mushroom fruiting body samples

Chemical substance	Concentration (range, m/kg)	Mean concentration	Standards range and assessment	
			MRL[1] (mg/kg)	result compliance[2]
Chlormequat chloride	0.005–0.016	0.009	0.9	compliant
Mepiquat chloride	0.005–0.051	0.015	0.09	compliant
Chlorate	0.020–0.55	0.167	0.7	compliant
Cyromazine	0.12–1.47	0.51	10.0	compliant
Melamine	0.013–0.56	0.045	2.5	compliant
Metrafenone	0.007–0.087	0.022	0.5	compliant
Prochloraz-Mn	0.006–0.098	0.028	3.0	compliant

Explanations – see Table 7

The same results concerning analysis of occurrence of pesticides in organic and conventional food was preformed by Montiel-León et al. [2019].

Statistical analysis revealed significant differences in pesticide residues between organic and conventional fruiting bodies. Conventional mushrooms exhibited a significantly higher frequency of mepiquat chloride and chlormequat chloride. Other fungicide, such as azoxystrobin, benzovindiflupyr, folpet, fthalimide, 2-phenylphenol, tebuconazole, and thiamethoxam, were detected at relatively low levels (0–1.4%) in both groups of samples. Among organic mushrooms, 40.1% of samples were free from residues, in contrast to the lack of residue-free samples in the conventional ones (Table 10).

Residues of prochloraz and metrafenone, commonly applied in *A. bisporus* cultivation, were frequently detected in conventional mushrooms from 2021 to 2023.

Table 10. Number of samples with chemical residues in organic and conventional mushroom fruiting bodies over a period of four years

Substances analyzed	Mean number of mushroom samples with residues (%)	
	organic	conventional
Chlormequat chloride	5.7 ±6.8 B	33.5 ±10.6 A
Mepiquat chloride	32.6 ±30.3 B	92.4 ±4.1 A
Azoxystrobin	0.0 ±0.0 A	1.4 ±2.8 A
Benzovindyflupyr	0.0 ±0.0 A	1.4 ±2.8 A
Chlorate	13.3 ±9.4 A	18.4 ±12.3 A
Folpet	0.0 ±0.0 A	1.4 ±2.8 A
Fthalimide	0.0 ±0.0 A	1.4 ±2.8 A
2-phenylphenol	0.0 ±0.0 A	1.4 ±2.8 A
Tebuconazole	0.0 ±0.0 A	1.4 ±2.8 A
Tiametoxam	0.0 ±0.0 A	1.4 ±2.8 A
Without residues	40.1 ±27.8 A	0.0 ±0.0 B

Explanations – see Table 5

This finding is consistent with results from other studies conducted by Carrasco et al. [2017] and Schusterova et al. [2023]. Nevertheless, in 2024 only a single sample contained prochloraz–Mn. However, the authorisation for its use in mushroom cultivation was withdrawn on 1 October 2023. Furthermore, in 2023, one sample contained carbendazim residues, although this substance is no longer authorised for mushroom protection [Commission Implementing Regulation 542/2011]. Another sample showed a twofold exceedance of the permitted level of 2-phenylphenol. A study by Li et al [2022] revealed that the primary pesticides found in edible fungi samples were carbendazim, acephate, procymidone, prochloraz, and aldicarb sulfone. According to Commission Implementing Regulation No 542/2011, the authorisation of carbendazim as an active substance for use in plant protection expired on 30 November 2014. Consequently, products containing carbendazim were removed from the register in June 2016. In 2024, thiamethoxam was detected in one sample of mushroom fruiting bodies at a concentration of 0.03 mg kg^{-1}, which exceeds the maximum permitted level (0.01 mg kg^{-1}). Moreover, the use of thiamethoxam was authorised until 19 December 2018 [Commission Implementing Regulation 2018/785, Commission Implementing Regulation (EU) 2022/801]. Additionally, it was determined that in 2023 and 2024, a significantly lower number of samples (11 out of 43) contained chlormequat chloride residues compared to the years 2021 and 2022 (23 out of 57).

Chlorate residues were frequently detected in mushroom samples. The analysis revealed that 14% of organic samples and 19% of conventional samples contained these substances. This occurrence is likely associated with the use of disinfectants during the cleaning of mushroom growing chamber or water disinfection for irrigation purposes in mushroom cultivation [Kettlitz et al. 2016]. Kettlitz et al. [2016] obtained similar conclusions in their study on the chlorate residue in food. They revealed that 50.5% of the food samples contained chlorate above 0.01 mg kg^{-1}. However, this was not due to the use of chlorate as a pesticide. Instead, it was mainly due to the occurrence of chlorate as an unavoidable by-product of disinfection. Furthermore, Gómez-Ramos et al. [2020] indicated chlorate residues in a large part of the samples of tested organic food. According to Zhang et al. [2023], high levels of chlorate were also detected in *Agaricus blazei* mushrooms. The potential pathways through which mushrooms can be contaminated with chlorate are not yet finally elucidated. However, it

is reported that chlorate residues arise in many cases by using chlorinated water either for irrigation in the field or post-harvest for various food processing, i.e. washing of equipment and surface disinfection in mushroom growing chamber [Commission Regulation 2020/749, European Food Safety Authority CONTAM Panel 2015].

CONCLUSION

The results obtained provide awareness of the presence of pesticide residues in organic mushrooms, originating from the cultivation substrate. Furthermore, no residues were detected in 40.1% of the organic fruiting body samples, while 32.6% of the samples contained mepiquat chloride. Only 34.1% of the organic substrate samples were free from chemical residues. Chlormequat chloride was detected in 11.1% of the samples. The other samples were mainly contaminated with chlorates, folpet, and phthalimide, not exceeding the maximum residue limits (MRLs).

In contrast, all mushroom samples from conventional cultivation contained pesticide residues, generally below the established MRLs. The most frequently detected residues were mepiquat chloride and chlormequat chloride, followed by prochloraz, chlorate, and melamine. However, one mushroom sample exceeded the permissible concentration of 2-phenylphenol, and two other samples contained residues of unapproved substances, such as carbendazim and thiamethoxam. Moreover, the level of thiamethoxam residue exceeded the maximum acceptable limit. The present study confirmed that conventionally cultivated mushrooms were associated with a higher occurrence of residue, while organic production resulted in a greater proportion of residue-free samples.

The results of this study indicate the need for continuous monitoring of white button mushrooms from both organic and conventional cultivation with regard to the presence of plant protection product residues. Moreover, the presence of residues in organic mushrooms does not preclude them from being classified as organically produced. Although the number of analyzed samples was limited, such monitoring is essential to ensure the safety and quality of agricultural products.

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