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A Comprehensive Review of Pests, Pesticides and Residue Detection Techniques in Mushroom

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TB and BK conceptualized and designed the research work. Authors TB and BK Investigated the study. Author TB wrote original draft. Authors TB, SM, SK and SC wrote, reviewed and edited the manuscript. Authors BK, SM and SK, CN, TD supervised the study. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Mushrooms are valued for their dietary benefits and medicinal properties, being rich in proteins, minerals, vitamins, fiber, and essential amino acids. They offer significant health benefits, including the prevention of hypercholesterolemia and cardiovascular diseases, and possess antitumor, antiviral, antihypertensive, anti-inflammatory, and immunomodulating properties. Global mushroom production has seen substantial growth, particularly in Asia. However, mushroom cultivation is challenged by various insect pests, leading to significant crop losses. Pesticides are frequently

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applied to control pests in mushroom cultivation, but their use can lead to the presence of pesticide residues in the mushrooms, raising concerns about food safety. To detect these low levels of residues, multiresidue analytical methods are crucial. Among these, the QuEChERS method stands out as the most commonly used due to its sensitivity, selectivity, and accuracy in determining multiple pesticide residues simultaneously. These methods are capable of identifying various pesticide compounds in a single analysis, effectively addressing the specific biological characteristics of mushrooms. The maximum residue levels (MRLs) for pesticide residues in mushrooms differ, with strict guidelines enforced by regulatory authorities. This paper provides a comprehensive review of the pests that affect mushroom cultivation, the pesticides utilized, and the analytical techniques developed for the detection of pesticide residues in mushrooms.

Keywords: Mushroom; pests; pesticides; QuEChERS; multuiresidue.

1. INTRODUCTION

Mushrooms are well known for their dietary benefits and as a significant source of bioactive compounds with medicinal properties [1]. Mushrooms are considered a valuable supplement to cereals due to their high content of proteins, minerals, fiber, and essential amino acids. They are rich in protein (2-40%), fat (2-8%), carbohydrates (1-55%), and fiber (3-32%). Additionally, mushrooms provide vitamins (1.4-2.2 mg of thiamine, 6.7-9 mg of riboflavin, 21.1-33.3 mg of pantothenic acid, and 1.2-1.4 mg of folic acid), minerals (such as aluminum, iron, manganese, magnesium, zinc, and selenium), and ash (8-10%) [2]. Their low carbohydrate content makes them beneficial for preventing hypercholesterolemia and cardiovascular diseases [3]. Moreover, certain mushroom species possess antitumor, antiviral, antihypertensive, anti-inflammatory, and immunomodulating properties [4]. The popularity of mushrooms is growing, with increased consumption of both fresh and processed varieties in the market. Over the past 20 years, global mushroom production has increased at a compound annual growth rate (CAGR) of 8.26%. Among continents, Asia has experienced the highest CAGR at 8.97%, followed by Africa with a CAGR of 6.31% [5]. Since 2000, global mushroom production has increased more than fivefold, reaching 44 million tonnes as of 2023 [6]. Asia dominates production, contributing 95% of the total, followed by Europe with 3% and the Americas with 1%. Leading countries in fresh mushroom production include China (93%), Japan (0.01%), and Poland (0.01%). India ranks sixth, with around 0.24 million tonnes of production according to data of FAO, 2023. Though domestic updates suggest it has reached 0.31 million tonnes. Despite significant advancements in China, the compound annual growth rates (CAGR) for fresh mushroom production in China (9.23%) and India (8.58%) over the last 20 years are comparable, reflecting encouraging growth for the Indian mushroom industry relative to the global leader. In regions like Europe and the USA, white button mushrooms are predominantly cultivated on a commercial scale. However, in Asian countries such as China, a variety of mushroom species are also commercially produced. Mushrooms are exported to UAE, Russia, Netherland, Germany, Switzerland, U.K, Denmark, Sweden and other countries from India [7].

Mushrooms are susceptible to a range of insect pests that can cause substantial crop losses [8]. In order to prevent this problem, pesticides are commonly used during mushroom cultivation. The indoor cultivation environment, characterized by limited light and high moisture, creates ideal conditions for pests and diseases, which can significantly impact yield and quality [9]. Key pests include sciarids (*Lycoriella* sp.), phorids (*Megaselia* sp.), springtails (*Lepidocyrtus* sp.), and mites (*Tyrophagus* sp.) [10]. Flies are particularly problematic, contributing to yield losses of up to 60-70% globally [11]. Their larvae damage the developing fruiting bodies and mycelial front, often rendering mushrooms unmarketable. The sciarid fly (*Lycoriella* sp.) is notably detrimental, leading to yield losses of up to 40% [10]. Additionally, phorid flies act as vectors for the pathogen, *Verticillium fungicola* var. *fungicola.* Improper pasteurization of compost and casing becomes a major source of fungal and bacterial infections. The attraction of mushrooms to decaying fungi also attracts flies, complicating pest management [11]. To prevent yield loss, pesticides are widely utilised mainly throughout the growing process as well as after cultivation [12]. Uncontrolled implementation of these pesticides leads to pesticide residues on agricultural products [13]. It was found that

residues of some of the insecticides such as chlorpyriphos, cypermethrin, methomyl and several pyrethroids occur in the mushrooms, soil and water [14]. Pesticide residue analysis was done in 145 samples of mushroom. Carbendazim was more frequently detected above the MRL [15]. Permethrin (50 g/ kg; MRL - 100 g/kg) and cypermethrin (200 g/kg; MRL - 500 g/kg) were found in shiitake mushroom and malathion (30 g/kg; MRL - 500 g/kg) was found in *Pholiota nameko* (nameko mushroom) samples [16]. Edible mushrooms are highly preferred and hence presence of detectable residues may pose threat to food safety and entry to international market. Therefore, it is important to monitor residues in mushroom to reduce the use of pesticides and improve the quality.

Highly selective, sensitive and accurate analytical methods are needed to measure low amount of pesticide residues in mushroom. Multiresidue methods are efficiently used as there are large number of pesticides used and should be detected in one single run [17]. The traditional pesticide residue analysis is typically used to identify a single pesticide ingredient, whereas the multiresidue analysis method can be used to identify not only various pesticide ingredients, but also various ingredients of different pesticide categories. As a result, one of the topics of analytical chemistry that is being actively researched is the analytical technique for pesticide multi-residue [18]. Vegetables in general have biological characteristics that are extremely different from those of edible fungus. The reverse resistance of fungi is weak due to lack of surface protective structures like wax layers, etc.

The MRL level for pesticide residues in mushroom ranges between 0.001 mg/kg to 3 mg/kg depending on the pesticide [18]. It may even established higher limits like in Bromide ion compounds has MRL of 30 mg/kg. The EURLs are responsible for promoting new analytical methods by proper guiding and organising proficiency tests, supporting the creation which leads to certification of novel analytical methods. Guidance is provided to laboratories for the validation of procedures to analysing pesticide residues in food and feed by the Directorate for Health and Food Safety of the European Commission [19]. This instruction gives laboratories the freedom to choose their own procedures, which is advantageous for the ongoing improvement of analytical techniques. Laboratories that assess pesticide residues frequently operate under a quality management system like ISO/EC 17025. This paper gives overview of pests, pesticides and different analytical methods which were adopted in analysis of pesticide residues in mushroom upto today.

2. PESTS OF MUSHROOM

2.1 Insect Pests of Mushroom

2.1.1 International status

Mushrooms are more prone to insect pests mainly due to their flavor and cultivation practices, which cause tremendous yield loss. The presence of insect pests reduces quality, quantity, marketability and palatability of mushroom. [20] reported that phorid fly (*Megaselia* sp.) was the major pest in USA and made direct damage by feeding on mushroom and also indirectly by transmission of fungal pathogens. Sciarid fly (*Lycoriella* sp.) considered as one of the dominant insect pest of mushroom crop throughout the world which cause significant yield loss up to 40 per cent [9]. Compared to other mushroom species, the sciaird fly *Lycoriella mali* (Fitch) prefers the button mushroom, *Agaricus bisporus* (Lange) Imbach. Infestation was more in button mushroom due to high developmental period, size and survival rate [21]. Sciarid fly, *Lycoriella ingenue* (Dufour) was reported as dominant pests in North America and both *Lycoriella ingenua* and *Lycoriella castanescens* (Lengersdorf) in U.K. In other regions of the world, *Bradysia* sp. (Sciarid fly) reported as major pest of button mushroom causing serious damage, though it also reproduce on oyster and shiitake mushrooms [22]. Pyemotid or pygmy mites, often known as red pepper mites, usually feed on moulds like *Trichoderma, Monilia*, and *Humicola* sp. They were predominantly present during commercial button mushroom production, which reduces marketable quality [23]. The key pests of mushroom reported from Korea include Sciarid flies namely *Camptomyia corticalis* (Loew), *C. heterobia* (Mamaev), *L. ingeneua* (Dufour) [24]. In Pakistan, the most destructive pest causing serious threat to mushroom cultivation is phorid fly, *Megaselia halterata* (Wood*).* The excreta of the larvae deteroite the quality of mushroom. They primarily act as vectors of pathogens especially *Verticillium* sp. [25]. Outbreak of pomace fly (*Drosophila* sp.) was severely noticed during 4th to 5th month of

mushroom cultivation [26]. Sciarid flies, *Lycoriella. auripila* (Winnertz), *Lycoriella. ingenua* (Dufour), *Lycoriella. solani* (Winnertz), phorid flies, *Megaselia. halterata* (Wood) and black flies - *Scaptopse notata* (Linnaeus) were the pests of mushroom reported from Turkey. *L. solani* was the common and cause more infestation from March to May and September to late November compared to other sciarid species [27]. Button mushroom and oyster mushroom were attacked by the cecid larvae, *Mycophila speyeri* (Barnes), *Heteropeza pygmaea* (Winnertz) and *Mycophila barnesi* (Edwards) in USA. The larvae feed on the exterior or at the point where the stipe and gills meet. They may cause a direct yield loss or reduction in the amount of fresh or processed marketable produce [28]. *Bradysia impatiens* (Johannsen), *B.ocellaris* (Cosmstock), *Lycoriella agraria* (Felt), and *L. ingenua* were the sciarid flies that were described from Australia. Larvae of sciarid flies mainly feeds on the growing mycelium and through tunneling it destroys sporophore primordia [29]. Throughout the world, mushrooms were primarily attacked by three dipteran pests such as sciarid flies, *Lycoriella mali*, *L. auripila*, *L. solani*, phorid fly, *Megaselia halterata* (Wood) and cecid flies, *Mycophila speyeri* (Barnes), *Mycophila. barnesi* (Edwards), *Hetropeza pygmae* (Winnertz). Minor pests of mushroom were pomace fly (*Drosophila* sp.), springtails (*Lepidocyrtus* sp.) and mites, *Tarsonemus mycelophagus* (Hussey), *Pymephorus* sp. *and Tyrophagus* sp. [10]. Phorid fly, *M. halterata* has recently turned from minor to major pest and caused mushroom yield reduction from 10-40 per cent in USA, U.K and Turkey. *Megaselia flavinervis* (Malloch), *Megaselia agarici* (Lintner), *Megaselia iriguoiana* (Felt) and *Megaselia bovista* (Gimmerthal), *Megaselia sandhui* (Disney) and *Megaselia tamilnaduensis* (Disney) were other phorid fly species which were reported in mushroom growing areas worldwide [30].

2.1.2 National status

Sciarid fly, *L. castanescens* and phorid fly (*Megaselia sp.)* were the threatening insect pests of mushroom in Kashmir valley [31]. First report of sciarid fly (*Lycoriella* sp.) on oyster mushroom was recorded during the month of April-May, 2012 in Assam. A single larva can cause damage around 20-35 per cent within 3-4 days of infestation. In severe infestations, 70-80 per cent of the fruit bodies in mushroom were damaged [32]. In India, mushrooms were dominantly attacked by pests such as: Sciarid fly, *Bradysia paupera* (Tuomikosi), *Bradysia. tritici* (Coquillett), *Lycoriella auripilla* (Winnertz), phorid fly (*Megaselia* sp.) and cecid fly, *Heteropezina cathistes* (Pritchard) which cause drastic reduction in mushroom yield. Springtails, *Lepidocyrtus cyaneus* (Tullberg), *Xenylla* sp. *Seria iricolor* (Say), mites (*Tyrophagus* sp., *Pygmephorous* sp.) and beetles, *Staphylinus* sp., *Alphitobius laevigatus* (Fabricius) and *Scaphisoma nigrofasciatum* (Pic) were found to cause 5-10 per cent damage. Minor pests such as spring tails (*Xenylla* sp., *Seria iricolor*) mainly damages mycelium and sporophore in the compost of both oyster and button mushroom. Besides it feeds on fruiting bodies and gills of mushroom. Nematode species such as *Aphelenchoides* sp., *Seinura* sp., *Tylenchids* sp. and saprophagous nematodes genera like *Rhabditis, Caenorhabiditis, Diplogaster*, *Acrobiloids* and *Panagrolaimus* were also infest mushroom [33]. Singh [34] reported that mites (*Tyrophagus* sp.) infest mushrooms starting from spawning to harvest and dipteran flies (sciarids and phorids) act as vectors for mites infestation in cultivated mushroom. Button mushroom is majorly attacked by insect pest belonging to order diptera and coleoptera in the Himalayan region. Spring tails and mites were also frequently encountered. Dipteran flies mainly are sciarid fly and phorid fly. *Lycoriella* sp. (Sciarid fly) has been identified in several countries that grow mushrooms, it has recently been documented in Himachal Pradesh. Additionally, *Megaselia* sp. (Diptera: Phoridae) was only found in two places. *Cyllodes indicus, Scaphisoma nigrofasciatum, Staphylinus sp. and Spondotriplax pallidipes* were four genera of beetles that were commonly found [35]. Incidence of sciarid fly (*Lycoriella sp.*) and phorid fly (*Megaselia* sp.) was frequent in button mushroom cultivation when compared to oyster and milky mushroom [36]. Two closely related fungus gnat species, *Bradysia ocellaris* C. and *Bradysia impatiens* J., have been reported in glasshouse insect cultures, emerging from plant soil. These gnats compete with target insects for food resources and act as vectors for pathogens, thus posing a considerable threat to the integrity of insect cultures [37].

2.2 Diseases of Mushroom

Apart from pest species, diseases were also a threat in mushroom cultivation. The prevalent bacterial disease affecting white button mushrooms is bacterial blotch, *Pseudomonas*

tolaasii (Tolaas). Interstinal necrosis was the bacterial disease caused by bacteria *Ewingella americana* (Grimont). Fungal diseases were dry bubble, *Verticillium fungicola* (Preuss), wet bubble - *Mycogone perniciosa* (Magn), cob web *Cladobotryum dendroides* (Bull), false truffle *Diehliomyces microspores* (Deihl and Lambert), white plaster mould *Scopulariopsis fimicola* (Garcha) and brown plaster mould *Papulaspora byssina* (Hots) were the fungal diseases which cause damage to button mushrooms consistently [38]. White button mushrooms were frequently affected by the fungi *Verticillium* sp, *Cladobotryum* sp., *Trichoderma harzianum* (Rifai), *Trichoderma viride* (Persoon). Green mould was the most affecting disease over past 20 years, which was due to pathogen *T. aggressivum* (Samuel). Wet bubble, dry bubble and other significant fungal diseases were detrimental to the cultivation of button mushroom in Turkey [39]. The most prevalent viral disease, dieback was brought by different virus types. These illnesses pose a serious danger to the multimillion-dollar mushroom industries because they could result in losses of up to 100 per cent [39].

3. PESTICIDE USAGE PATTERN IN MUSHROOM

Due to attack of different pests in mushroom, it has become necessary to use pesticides to maintain yield. Since cultivation practices is having different temperature and humidity that introduces a wide range of pests. In order to solve these issues, growers are turning to use more pesticides recently.

Diflubenzuron showed 90-96 per cent effective control against sciarid larvae but could not avoid reduction of mushroom yield up to 7-8 per cent [40]. The quantity of immature sciarids during the first cropping period was reduced by 99 per cent by adding diazonin (25 mg a.i/kg) to mushroom compost, followed by an additional application of diflubenzuron (1 g a.i/m²) to the casing layer. Treatment with diazonin and diflubenzuron together in the first flush, reduced the appearance of sciarids [15]. Stoddart [41] recommended to use bendiocarb and diflubenzuron either drenched onto casing or incorporated in compost of mushroom which showed effective control around 50 to 70 per cent for flies, spiders, bugs, mites and larvae of insect pests. Malathion 0.05 per cent and dichlorvos 0.5 per cent were recommended as effective control for mushroom pests and applied in composts [42]. Aldrin, DDT, benzene hexachloride and thionazin was incorporated into the compost at both 100 and 500 µg/g and showed effective control. Bendiocarb was shown to be more effective in the range of 10 to 30 µg/g for sciarids when applied in casing. Diazonin when incorporated into the compost at spawning period showed 96 per cent control of sciarid flies @ 100 mg/kg [43]. Shin [25] recommended use of deltamethrin, spinosad, trichlorfon, malathion and permethrin for the control of phorid flies in mushroom and conducted study to know effective insecticides against phorid flies in mushroom. The insecticides were deltamethrin 2.5 EC @ 0.4 ml/l, spinosad 240 SC @ 0.4 ml/l, spinoteram 120 SC @ 0.2 ml/l, trichlorfon 80 WP @ 6.8 ml/l, malathion 57 EC @ 7.5 ml/l and permethrin 0.5 per cent @ 0.25 ml/l. The emergence and damage rate were reduced due to use of above insecticides in mushroom cultivation. Compared to other insecticides, spinosad was shown effective by maximum reduction of adult emergence and less damage rate by phorid flies [44] evaluated five fungicides such as carbendazim, bitertanol, hexaconazole, captan and mancozeb in vitro against greenmould disease and found that the maximum control of green mould disease occurred in carbendazim (90.8%), followed by bitertanol (40.0%), captan (36.6%) and hexaconazole (16.1%). Mancozeb had the least amount of control (11.7%).

Fungicides including prochloraz, benomyl, carbendazim and mancozeb were used for the control of fungal diseases. Prochloraz was the only one that is registered in Turkey for cultivated mushrooms [45]. Mortality of mushroom pests were observed maximum when treated with imidacloprid (88.88%) followed by dichlorvos (88.09%), thiamethoxam (61.10%) and malathion (35.71%) at 0.01 per cent [46]. Dicofol 50 EC @ 1-2 ml/l was to be sprayed at regular interval of time on the wall of mushroom house and also in compost for the control of mites. Control of springtails can be done by spraying malathion 0.05 per cent as disinfection spray [47]. Tian et al. [48] recommended use of pesticides such as fenpropathrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, flucythrinate, fenvalerate and deltamethrin for effective control of sciarid and cecid flies. Application of permethrin dust (10 g a.i./kg) had controlled mushroom flies (sciarids, phorids and cecids) effectively. Aerosol formulations containing the insecticide active component pyrethrin or a mixture of pyrethrin and

resmethrin may be sprayed or fumigated within mushroom manufacturing houses. It should be applied with "cold fogging" tools. A spray application with a maximum concentration of 700 ml of product per 100 litres of water should be used to apply a product containing 10 g/l pyrethrins for the control of mushroom flies [49].

4. COMMON INSTRUMENTAL TECHNIQUES FOR ANALYSIS

4.1 Sample Preparation

About 600 gm - 1 kg of mushroom samples were chopped, mixed and homogenized [50,16,48,14]. In that, only 100 gm is taken for as sub-sample for test purpose. Samples were stored at -18 to 20 ºC in freezer for further analysis.

4.2 Sample Extraction

The usage of the separation technique depends on type of sample and its any potential matrix interfering during extraction. The most common method used in mushroom for pesticide analysis was LC-MS/MS [50,51,52,53,48,14]. It is necessary to take into account the physicochemical characteristics of the analyte and also the polarity of the pesticide. The complexity of sample treatment has been reduced while the accuracy and precision of the analysis have grown because to advancements in analytical techniques and extraction procedures. Using acetonitrile and petroleum ether, a multi-residue approach for analyzing organochlorine pesticides in food was created in 1963. A method based on acetone followed by dichloromethane and petroleum ether partitioning and clean-up with Florisil to be able to analyze more polar pesticides than the organochlorine group. The Dutch Food and Consumer Products Safety Authority-Food Inspection Service also created an extraction technique based on acetone in 1983, and it was regularly used for pesticide monitoring for more than 25 years. In 1989, the Swedish National Food Administration created an analytical procedure involving ethyl acetate and a clean-up step utilizing gel permeation chromatography. Since ethyl acetate has a lower polarity index (4.4) than acetone (5.1), polar insecticides partition less readily in ethyl acetate. The water phase is heavily supplemented with anhydrous sodium sulphate (Na_2SO4) in order to force the polar insecticides into the organic solvent. A novel method based on acetonitrile extraction and clean-up employing dispersive solid phase extraction (dSPE) with primary and secondary amines (PSA) and octadecylsilyl (C_{18}) was introduced in 2003 by Anastassiades *et al*. This sample treatment method was dubbed QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) by the researchers. This technique gained popularity since it used fewer conventional analytical stages, solvents and glassware. As a result, two reference methods were published: the first was CEN15662 by the European Committee for Standardization, which used acetonitrile and a citrate buffer during the extraction, and the second was "Method 2007.01" by AOAC International, which used acetonitrile and an acetate buffer during the extraction. Many methods have been published for the analysis of pesticides in mushrooms based on QuEChERS methodology. During the year from 2013 to 2022 [50,51,52,53,48,14], method standardization was carried out by using QuEChERS method (ACN as main solvent) along with LC-MS/MS mostly. Overview of published literatures are presented in Table 1. In general, common procedure to analyse a large number of pesticide residues in mushroom uses Acetonitrile as organic solvent (Extraction solvent).

4.3 Clean-up Extraction Method

The preliminary extraction using organic solvents is typically followed by a clean-up step. Various methods for this process are described below.

The most common clean-up agents used in extraction mushroom is PSA, GCB, C_{18} and $MgSO_4$ [52,53,48,14]. Residue behavior of six pesticides in button mushroom was analyzed using UPLC-MS/MS after performing extraction by QuEChERS method. A 10 g of sample was weighed into a 50 ml centrifuge container. Two ml of water and 10 ml of acetonitrile were then added for LC-MS/MS whereas for GC-MS/MS detection 10 ml of ethyl acetate was used followed by addition of 4 g of $MgSO_4$ (LC-MS), 3 g $MgSO_4$ (GC-MS) and 1.0 g of NaCl were then added. The containers were immediately sealed, vigorously spun for three minutes, and then centrifuged at 3500 RCF (Relative Centrifugal Force) for five minutes. 1.5 ml of the acetonitrile (upper) layer was decanted in a centrifuge tube containing 100 mg of anhydrous $MgSO_4$ and 50 mg of PSA. For GC analysis, 20 mg of florisil was used. After being spun for one minute, the

Table 1. Overview of published multi-residue methods for the analysis of pesticides in mushroom. Recovery is expressed in percentage. Repeatability is expressed as relative standard deviation percentage

Matrix	Pestcides	Extraction	Clean up	Method	Column	LOQs	LODs	Linear range	Recovery (%)	Reference
Fresh mushroom (Crimini, shiitake and oyster) Substrates	Pyriproxifen, avermectin and diflubenzuron	LPE with ACN	SPE with $MgSO_4$ +PSA	UPLC-MS/MS	C-18	0.052 to 5 µg/kg	0.016–1.5 mg	0.5, 1 or 5 to 5000 µ g/l	78.1-112.5	[50]
Shiitake mushroom, enoki mushroom and black edible fungus	187 pesticides	LPE with ACN	SPE with GCB	LC-MS/MS	C-18	0.02–170 µg/kg	0.01 to 85 µg/kg	0.05–33 920 µg/kg	70-118	[51]
Edible mushrooms and substrates (oyster, shiitake, eryngii and crimini)	Phoxim, chlorpyrifos and pyridaben	LPE with ACN	$MgSO_4$ +PSA	UHPLC-MS/MS	Waters ACQUITY UHPLC BEH C18	1-10 µg/kg	1 to 10 µg /kg	0.01 to 1.0 µg/kg	75.5 to 98.4	[52]
Edible fungus	28 OP compounds	LPE with ACN	SPE $MgSO_4$ +PSA	GC-MS	DB-1701MS fused-silica	1.0 to 10 µg/kg	0.3 to 3.0 µg	3.0 to 1000 µg/kg	85.26 to 100.21	[55]
Mushrooms (*Lentinula edodes*)	62 Pesticides	LPE with ACN	SPE with PSA + C18 + GCB + $MgSO_4$	GC-MS/MS	DB-5MS UI colum	0.625 to 20 µg/kg		10, 50, and 100 g/kg	75.4 to 117.1	[16]
Button, enoki, oyster, elm oyster and shiitake mushroom	clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam	LPE with ACN	PSA+C18+ GCB	UHPLC-MS/MS	Agilent ZORBAX Eclipse Plus C18 column	0.1 to 2 µg/kg	0.03 to 0.7 µg/kg	10, 50 and 500 µg/kg	73.9–89.5	[53]
Edible mushrooms	flubendiamide, chlorantraniliprole, cyclaniliprole, tetrachlorantranilip role and cyantraniliprole	LPE with ACN	PSA+C18+ GCB+ $MgSO_4$	HPLC-MS/MS	Agilent Poroshell 120 EC-C18 column	5 µg/kg	0.05-2 µg /kg	0.005 to 0.5 mg/l	73.5–110.2	[54]
Oyster mushroom, shiitake mushroom, eryngii mushroom, crimini mushroom,	ten pyrethroid (Bifenthrin, fenpropathrin, cyhalothrin,	LPE with ACN	SPE with PSA+C18+ $MgSO_4$	GC-MS/MS.	Agilent Technologies Capillary Column HP-	0.051 to 5.57 µg/kg	0.015 to 1.67 µg/kg	10, 100, and 1000 µg/kg	72.8 to 103.6	[48]

Matrix	Pestcides	Extraction	Clean up	Method	Column	LOQs	LODs	Linear range	Recovery (%)	Reference
enoki mushroom and bunashimeji mushroom	permethrin, cyfluthrin, cypermethrin, flucythrinate, tau-fuvalinate, fenvalerate and deltamethrin				5MS phenylmethyl siloxane fused-silica capillary analytical column					
Enoki, button, oyster and shiitake	180 pesticides	LPE with ACN	SPE with PSA + C18	UPLC-MS/MS	ACQUITY UPLC BEH C18 (1.7 μm, 100 × 2.1 mm) column	0.02–170 μg/kg	0.01–85 μg /kg	0.05–33 920 μg /kg	70 to 120	[14]

materials were centrifuged at 3500 RCF for five minutes. A 0.22 µm nylon syringe filter was used to filter the prepared sample's top layer, which was then moved to an auto-sampler for analysis [50]. For determination of five neonicotinoid residues in mushroom, 10g of homogenized edible mushroom was taken in 50 ml conical beaker after which 1.0 ml of the working solution was added. The sample was left for 20 minutes to enable the solvent to evaporate. The extraction fluid contained 15.0 ml of acetonitrile and 5 millilitres of distilled water. Following that, the beaker was placed on an electric orbital shaking incubator and agitated for 15 minutes at room temperature for 150 rpm. The entire contents of the beaker were then filtered under vacuum into a 100 ml measuring cylinder that contained 5 g of NaCl. The cylinder was then manually shaken for about 2 minutes before being left on the table for 1 hour to enable phase separation. A 5 ml centrifuge tube containing 150 mg of anhydrous $MgSO_4$ and a specific quantity of sorbent was then filled with 1.5 ml of the acetonitrile phase and vortexed for 30 seconds before being centrifuged at 5000 rpm for 5 minutes. In order to prepare for UHPLC-MS/MS analysis, 0.6 ml of the supernatant was then removed from the container using an SGE glass syringe. It was then combined with 1.0 ml of the mobile phase (20% methanol + 80% ultrapure water containing 0.1% formic acid) and passed through a 0.22-µm nylon syringe filter [53]. Five diamides such as flubendiamide, cyclaniliprole, chlorantraniliprole, tetrachlorantraniliprole and cyantraniliprole residue extraction in edible mushrooms was carried out by QuEChERS method. An aliquot of 10 g homogenized sample was taken in 50 ml of centrifuge tube. Acetonitrile (10 ml) was used as solvent for extraction. Next, salt mixture containing 1 g of Nacl and 4g of $MgSO_4$ was added followed by votexing for one minute and centrifugation done at 2077 rpm for 5 minutes. In the cleanup step, for oyster mushroom, enoki mushroom, bunashimeji mushroom and crimini mushroom (20 mg PSA and 30 mg C18), 20 mg PSA and 50 mg C_{18} for shiitake mushroom and eryngii mushroom was added. To all samples 150 mg of $MgSO_4$was too added. The supernatant was decanted (0.9 ml) into an injection vial with a 0.22 µm organic syringe filter for HPLC-MS/MS analysis [54]. Estimation of 180 pesticide residues in mushroom was performed by applying QuEChERS method. A 2 g of homogenized sample was taken in 50 ml of centrifuge tube. To that, 10ml of acetonitrile (ACN) was added then vortexed for 1 minute and below 40°C sonification was done for 15 minutes. Later, 1 g of NaCl, 4 g of $MgSO_4$, 0.35 g of trisodium citrate dihydrate and 0.2 g of disodium citrate were added. Again vortexed for 1 minute and centrifugation was done at 20,000 rpm. C18 and PSA was used for solid phase extraction. The solid phase extraction device was used to load 5 ml of the extract, which was then passed through the column before being collected. The elution solvent, which combines the sample extract and eluting solution, was 20 ml of the ACN: toluene (3/1, v/v) solvent system. Using turbo evaporator at 40°C, 10 mbar, and 50 minutes, the solution acquired through SPE extraction was concentrated to 1 ml of enriched solution. After refinement, one millilitre of the extract was dried using nitrogen gas flow at one degree, then redissolved in one millilitre of ACN:H_2O. Finally, the sample filtered by a 0.22 µm PTFE barrier and injected to UPLC-MS/MS [14].

4.4 Method Validation Parameters

Multiresidue analysis in mushroom was carried out in many countries such as China, Japan, Turkey, Veitnam, U.S.A, Poland etc. Method validation is done to assess parameters such as sensitivity, precision, linearity and recovery. QuEChERS method is sensitive, selective and accurate for determining multiple pesticide residues, so it is the mostly used method. [50] studied detection of pesticide residues (pyriproxyfen, diflubenzuron, and avermectin) in different mushrooms and their substrates using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) by Ultra-Performance Liquid Chromatography tandem Mass Spectrometry (UPLC-MS/MS). Relative Standard Deviation (RSD) values for all analytes were below 11.8 per cent, with average recoveries ranging between 78.1 and 112.5 per cent. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were 0.016 to 1.5 g/kg and 0.052 to 5 g/kg, respectively. A quick and accurate Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) method was applied for the simultaneous determination of 187 pesticide residues in shiitake mushroom, enoki mushroom and black edible fungus. The linear correlation coefficient r^2 was ≥ 0.990 for each pesticide's linear range. Recovery rates varied from 70 to 118 per cent at both low and high fortification levels. The RSD for 169 pesticides shown below 25 per cent which accounts for 90 per cent of the total. LOD ranged from 0.01 to 85 µg/kg for 165 pesticides [51]. The

determination of prochloraz residues were estimated using QuEChERS method combined with Gas Chromatography Electron Capture Detector (GC-ECD). This method has shown effective linear relationship with r^2 (0.999) for concentration 52.9 to 1000 µg/kg. The LOD and LOQ were 15.9 µg/kg and 52.9 µg/kg respectively. Mushroom samples had shown recovery of 100.6 ± 1.6 per cent for 250 µg/kg and 105.6 ± 3.9 per cent for 1000 µg/kg [45]. Tian [52] developed modified QuEChERS method for detection of phoxim, chlorpyrifos and pyridaben residues in edible mushrooms and substrate using UHPLC-MS/MS and Electro-Spray Ionisation source operating in the positive mode (ESI+) to identify the target molecules. For the three compounds, LOQ were 10 µg/kg, and LOD varied from 1 to 10 µg /kg with correlation values >0.994 achieved, using calibration curves of standards ranging from 0.01 to 1.0 µg/kg. Recovery of 75.5 to 98.4 per cent for concentration 0.01-0.25 mg/kg was noted. A new analytical method was developed using Gas Purge Micro-Syringe Extraction (GP-MSE) and Gas Chromatography-Mass Spectrometry (GC-MS) for detection of 28 organophosphorus pesticide residues in edible fungus. Organophosphorous insecticide showed recovery ranging from 85.26 to 100.21 per cent and RSD ranged from 1.6 to 6.9 per cent. The LOQ and LOD from 1.0 to 10 µg/kg and 0.3 to 3.0 µg/kg respectively [55]. Five neonicotinoide insecticides (clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) in mushroom (button, enoki, oyster, elm oyster and shiitake mushroom) were detected by UHPLC-MS/MS. The samples were processed using a modified QuEChERS procedure. For all analytes found in mushroom, LOD ranged from 0.03 to 0.7 µg/kg, whereas LOQ ranged from 0.1 to 2 µg/kg. The ranges of the mean recoveries for clothianidin, dinotefuran, imidacloprid, thiacloprid and thiamethoxam were 100.5-118.0, 73.9-89.5, 88.6-117.8, 72.9-121.8 and 98.9-117.2 per cent respectively with RSD less than 8.1 per cent for 10, 50 and 500 µg/kg concentration [53]. A modified QuEChERS technique for the simultaneous determination of flubendiamide, chlorantraniliprole, cyclaniliprole, tetrachlorantraniliprole and cyantraniliprole in edible mushrooms was developed applying HPLC-MS/MS. By plotting the peak area against the corresponding concentration at six calibration levels, 0.005 to 0.5 mg/l, the linearity for all the target chemicals in acetonitrile and matrix solutions was determined and found satisfactory ($R^2 \geq 0.99$). The LOD was 0.05-2 µg /kg and LOQ was 5 µg/kg. These pesticides had recoveries that were acceptable (73.5–110.2%) for concentration 5, 10 and 100 µg /kg. The RSD was below 12.7 per cent [54]. A technique was formed to simultaneously determine the presence of ten pyrethroid (Bifenthrin, fenpropathrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, flucythrinate, tau-fuvalinate, fenvalerate and deltamethrin) insecticide residues in six different edible mushrooms (oyster, shiitake, eryngii, crimini, enoki and bunashimeji). The samples were prepared using QuEChERS method combined with GC-MS/MS. Six different edible mushroom species were used to extract the 10 pyrethroid pesticides. At three levels (10, 100, and 1000 µg/kg), the average recoveries for the six different edible mushrooms varied from 72.8 to 103.6 per cent. The RSD were less than 13.0 per cent for both intraday and interday data. The LOQ were < 5.57 µg/kg [48]. [14] developed a highly sensitive and reliable approach to simultaneously identify 180 pesticides in different mushrooms (Enoki, button, oyster and shiitake) through the optimization of extract purification conditions. By combining QuEChERS extraction with a mixed mode of SPE (Solid Phase Extraction) cleaned up with various adsorbent materials after sample preparation, matrix effects were minimized. The LOQ varied from 2 to 5 µg/kg, which is significantly less than the MRLs set by the EU (10 - 50 µg/kg). Recovery rates ranged from 70 to 120 per cent and the RSD of repeatability and reproducibility were both less than 20 per cent. Three edible fungi (*Volvariella edodes*, *Volvariella volvacea*, *Flammulina velutipes*) were extracted with acetonitrile and purified using an improved QuEChERS method. GC-MS/MS (Agilent HP-5MS column) and LC-MS/MS (UPLC BEH C18 column) were used for external standard determination. Results showed good linearity (5-500 µg/L, $R^2 > 0.99$), with recoveries for 13 pesticides ranging from 68.2% to 119.8% and RSDs from 0.7% to 12.5% [55]. The methodology for pesticide residue analysis from sample preparation to analysis was shown in Fig. 1.

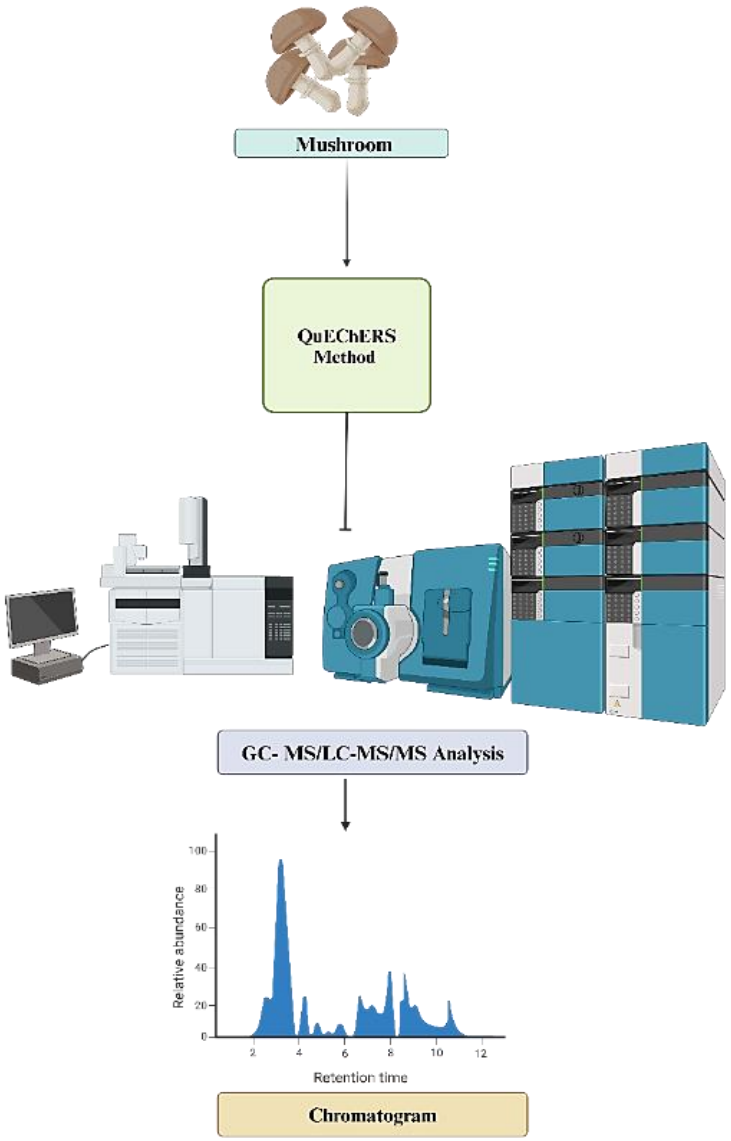

Fig. 1. Methodology for analysis of pesticide residue analysis in mushroom

5. MONITORING OF PESTICIDES

Pesticides are widely used in order to prevent and control diseases and insect pests during mushroom cultivation. Monitoring of pesticide residues in mushroom is thus becomes essential in order to prevent health hazards. The use of pesticides was reduced as a result of the application of integrated pest management concepts and excellent agricultural practices, with a tendency to reduce the most hazardous pesticides. A number of studies related to monitoring were conducted. In one of the study, [56] monitored 33 pesticide residues in a total of 4 fruiting body samples such as oyster mushroom, shiitake mushroom, button mushroom and fungus collected from the four provinces of Hubei, Zhejiang, Henan, and Fujian. The study showed that, 23.3 per cent of the samples exceeded the EU standard, 3.27 per cent of the samples above the Japanese standard while no sample exceeded the current level in China. Pesticides such as cymethrin, cyflumethrin, thiophenate methyl, methophos, methyl parathion, malathion and bethion were primarily found in the samples. Bethion was found in seven samples of button mushrooms, oyster mushrooms, and fungus. Cyflumethrin

was found in seven samples of oyster mushrooms. Thiophenate methyl was found in six samples of button mushrooms. Individual samples of button mushroom contained highly toxic pesticides like methamidophos, monocrotophos and methylparathion. [57] examined two mushroom species such as Xerocomus mushroom (*Xerocomus badius*) and Boletus mushroom (*Boletus edulis*) from Poland for the presence of organochlorine compounds using GC-MS. γ-HCH, DDT, and its metabolites (DDE, DDD) were found in all of the samples that were analyzed. Xerocomus mushrooms had the highest concentration of γ-HCH (0.125 µg/kg on average), while boletus mushrooms had a lower concentration of 0.11 µg/kg of mushrooms. [52] monitored 50 mushroom samples for presence of pesticide residues such as pyridaben, chlorpyriphos and phoxim. Mushroom samples such as oyster, shiitake, button mushroom were collected from Bejing local market of China. In button mushroom, pyridaben was found in the range of 0.08 to 0.015 mg/kg.In Western China, pesticide residue study was conducted to the know amount of pesticide residues in edible mushroom. Around 354 mushroom samples were brought from nearby markets and analyzed 53 pesticides using HPLC/MS/MS. Major pesticides which were found in the edible mushroom samples included carbendazim, acephate, procymidone, prochloraz, aldicarb sulfone etc. The detection rates for carbendazim, acephate and procymidone were 0.0002-2.7316 mg/kg, 0.0248-0.4985 mg/kg and 0.1807-0.3928 mg/kg respectively. Highest detection was noted with carbendazim (70.9%) followed by acephate (13%) and procymidone (7.3%). While the remaining samples of edible mushroom revealed one or more pesticide residues [58]. The presence of pesticide residues, their metabolites, and degradation products was examined in 49 edible mushrooms, including fresh, dried, canned, and frozen varieties. These samples, collected from Czech markets, were analyzed for 427 different analytes using QuEChERS extraction, followed by liquid and gas chromatography combined with tandem mass spectrometry. In total, 21 pesticide residues, metabolites, and pesticide synergists were found in measurable concentrations. The most frequently detected pesticide residues were prochloraz and its metabolites, metrafenone, and carbendazim. Two mushroom samples exceeded the maximum residue level. Additionally, the study investigated the behavior of 13 pesticides during the processing of fresh mushrooms [59].

6. FUTURE DIRECTIONS AND CHALLENGES

Regulation and Monitoring with Stricter regulations and enhanced monitoring are expected to be implemented to ensure that pesticide residues in mushrooms remain within safe limits. This may involve more frequent testing and tighter control measures. Alternative Pest Management, there is a growing interest in developing and adopting alternative pest management strategies, such as biological controls and integrated pest management (IPM), which could reduce reliance on chemical pesticides and thus minimize residues. Organic and Sustainable Farming, the trend towards organic and sustainable farming practices is likely to continue. Organic mushroom cultivation typically uses fewer or no synthetic pesticides, which can reduce the presence of pesticide residues. Advancements in Detection Technology such as more sensitive analytical methods, will enhance the ability to identify and quantify pesticide residues more accurately. This will aid in better compliance and safety assessments. Reducing matrix effect by using suitable clean-up agents and optimizing chromatographic conditions (e.g., adjusting column type, mobile phase composition, and flow rates) to improve separation and reduce matrix interference in techniques like HPLC or GC [60]. Consumer Awareness and Preferences, As consumers become more aware of pesticide residues and their potential health impacts, there will be an increasing demand for mushrooms grown with minimal pesticide use, potentially influencing farming practices. Research and Development, ongoing research into the effects of pesticides on mushrooms and their environment will contribute to understanding and managing residues better. This includes studying how pesticides break down and affect mushroom safety. Overall, the future will likely see a combination of regulatory, technological, and consumer-driven changes aimed at minimizing pesticide residues in mushrooms and improving food safety [60].

7. LIMITATIONS OF MULTIRESIDUE METHODS

The most common and efficient way to perform pesticide residue analysis for numerous different compounds involves using multi-residue

methods that can measure within the MRL range from 0.01 to 10 mg/kg [61]. However, these multi-residue methods cannot measure all pesticides with the necessary accuracy in a single run. The significant diversity in the chemical composition of these hundreds of pesticides hinders the application of a single strategy for their simultaneous analysis. Consequently, it is sometimes still essential to develop single-residue methods for the analysis of one pesticide or a few pesticides from the same chemical family. Examples include pesticides with high polarity or ionic characteristics. Another issue might be the low stability of specific pesticides during sample extraction. In the case of compounds with a high polarity or ionic compounds, new approaches based on LC have been proposed. They can be divided in three strategies: (i) the polarity of the analytes is reduced by derivatization of the analytes or by addition of an ion pairing substance to the mobile phase before analysis by RP-LC. This decreased polarity leads to an increased retention and more adequate peak shape [62]. (ii) Use of hydrophilic interaction liquid chromatography (HILIC) with carbon or ion exchange phases instead of reversed phases. Also this leads to an increased retention of the analytes [63]. (iii) Elimination of the separation technique and use of direct flow injection (FI) to MS/MS [64,65].

8. CONCLUSIONS

Mushrooms are recognized for their significant nutritional value and medicinal properties, making them a valuable addition to the diet and a popular supplement to cereals. Their rich content of proteins, minerals, vitamins, and bioactive compounds contributes to their growing market demand. However, the cultivation of mushrooms faces challenges, particularly from insect pests, which often leads to the extensive use of pesticides. While these pesticides are essential for controlling pests and diseases, their residues on mushrooms pose potential risks to food safety and marketability. The increase in mushroom production and international trade emphasizes the need for stringent monitoring of pesticide residues to ensure compliance with food safety standards. Advances in analytical methods, particularly multiresidue techniques, are crucial for accurately detecting and quantifying pesticide residues despite the complex matrix of mushrooms. These methods help mitigate the risk of pesticide contamination and support the export of safe, high-quality mushrooms. Efforts to reduce pesticide use and improve residue analysis are essential for maintaining food safety and enhancing the quality of mushroom products. By adopting effective pest management practices and utilizing advanced analytical techniques, the mushroom industry can address the challenges of pesticide residues and continue to thrive in both domestic and international markets.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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