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ARTICLE

Oxidative metabolism of quinazoline insecticide fenazaquin by *Aspergillus niger*

So-Hyun Joo[1] · Young-Soo Keum[1]

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Abstract Fenazaquin (4-(2-(4-*t*-butylphenyl)ethoxy)quinazoline) is a quinazoline insecticide, which contains a rare pesticidal toxophore, quinazoline. Its metabolic fate in animals and plants was previously reported. However, the microbial metabolism of the compound has never been studied. Microbial transformation is an important research area for the investigation of environmental safety issues of pesticides. *Aspergillus niger* was selected as a model soil fungus since it is ubiquitous in agricultural soils, with extensive genetic studies undertaken. Fenazaquin was rapidly metabolized by *A. niger* (half-life, $t_{1/2}$ = 0.6 day). 4-Hydroxyquinazoline and 4-*t*-butylphenethyl alcohol were identified as major metabolites from the cultures. Fenazaquin was also rapidly transformed into the same metabolites ($t_{1/2}$ = 0.1–0.5 day) under chemical oxidation (*m*-chloroperoxybenzoic acid). Among the several metabolic inhibitors, flavin-dependent mono-oxygenase inhibitor, methimazole yielded no inhibitory activity ($t_{1/2}$ = 1.6 day). Several cytochrome P450 inhibitors including piperonyl butoxide, ketoconazole, and myclobutanil were also tested. Piperonyl butoxide strongly reduced fenazaquin metabolism ($t_{1/2}$ = 58.7 days). However, ketoconazole and myclobutanil showed no activity even at fungi-toxic concentrations ($t_{1/2}$ = 1.2–4.3 days) with major metabolites similar to those of control experiments. The results suggest that oxidative metabolism of fenazaquin was catalyzed by specific cytochrome P450s, which are insensitive to azole fungicides. In addition, piperonyl butoxide was found to be one of the most promising synergists of pesticides, through cytochrome P450 inhibition.

Keywords *Aspergillus niger* · Cytochrome P450 · Fenazaquin · Metabolism · Quinazoline

Introduction

Quinazoline is a compound with a unique chemical configuration, which is found in a limited numbers of pesticides (e.g., fenazaquin, (4-(2-(4-*t*-butylphenyl)ethoxy)quinazoline). The insecticide can be used to control several mites [1, 2]. It inhibits mitochondrial electron transport chain at site I of the respiratory chain [2, 3]. Fenazaquin is a highly lipophilic compound (logP = 5.5) and hence is not translocated into plants from the agricultural environment [4]. However, few studies indicated the widespread contamination with this pesticide compared with other lipophilic pesticides [5]. Its half-life ranges from 3 to 60 days in soils and aqueous systems [6, 7]. Metabolic transformation of fenazaquin has been studied in a few animals and plants [4–6]. However, its fate in microorganisms has never been reported.

Pesticides in soil and aqueous environment are subjected to various degradative reactions, among which biotransformation is often one of the most significant determinants of degradation rates [8]. Because of their large biomass, populations, and genetic adaptation, bacteria and fungi are usually considered as major contributors in pesticide biodegradation [9, 10]. For example, several recalcitrant pesticides (e.g., organochlorine insecticides) were mineralized by white rot fungi [11–13]. In addition, numerous

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✉ Young-Soo Keum
rational@konkuk.ac.kr

[1] Department of Crop Science, Konkuk University, 1 Hwayang-dong, Gwanjin-Gu, Seoul 143-701, Republic of Korea

non-lignolytic fungi are also known to decompose structurally diverse pesticides [14–17]. Interestingly, several phytopathogenic fungi can rapidly metabolize pesticides [17–20]. For example, many *Aspergillus* and *Fusarium* species can efficiently metabolize organophosphate and carbamate insecticides [19, 20]. The *Aspergillus* species are well-known producers of extra-cellular hydrolase (e.g., lipases, proteinases, and other depolymerizing enzymes). Biodegradation of ester-type pesticides (e.g., organophosphates, carbamates, and pyrethroids) are usually catalyzed by hydrolases [21]. Aspergilli are also known to metabolize many other natural and synthetic compounds. For example, *Aspergillus niger* can metabolize numerous synthetic flavonoids via oxidative transformation [22]. The metabolites of these xenobiotics suggest that oxidative enzymes (e.g., cytochrome P450s, CYP) take a major metabolic role. Current genomic studies indicate that the *Aspergillus* genome contains more than 150 CYPs [23, 24]. Due to its metabolic versatility and genetic information, Aspergillus is a promising research tool for the study of microbial pesticide metabolism.

Rapid dissipation of pesticide is an important factor in food safety issues. However, it decreases the efficacy of pesticides, which results in reduced agricultural productivity. In addition, metabolic inactivation of pesticides is one of the most common mechanisms of pesticide resistance [25]. Inhibitors of pesticide metabolism are frequently included in commercial pesticide formulations (e.g., piperonyl butoxide).

In this study, fenazaquin was subjected to metabolic transformation by *A. niger*. Metabolic pathways were elucidated via chemical oxidation, specific enzyme inhibitor studies, and instrumental analyses.

Materials and methods

Chemicals

The following reagents were purchased from Sigma-Aldrich Korea Ltd (Seoul, Korea); including fenazaquin, 4-*t*-butylphenylacetic acid, piperonyl butoxide, methimazole, ketoconazole, myclobutanil, and *m*-chloroperoxybenzoic acid (MCPBA). 4-Hydroxyquinazoline and 2,4-quinazolinedione were obtained from Oakwood Products Inc (PA, USA). Other reagents for the syntheses were obtained from Alfa Aesar Korea (Seoul, Korea). Potato dextrose broth was obtained from BD Korea (Seoul, Korea). Solvent was HPLC grade or higher.

Synthesis of 4-*t*-butylphenethyl alcohol

4-*t*-Butylphenethyl alcohol was prepared by literature method [26].

Kinetic study of fenazaquin biotransformation by *A. niger*

Aspergillus niger KACC 45093 was kindly provided by the National Agrobiodiversity center, RDA-Genebank Information Center (Jeonju, Korea). The fungal seed culture was grown on a potato dextrose broth (PDB) for 3 days at 28 °C, 200 rpm. For the kinetics study, the mycelium from seed culture (0.2 g, fresh weight) was added to a freshly sterilized PDB (200 mL). Then, aliquots (0.5 mL) of fenazaquin solution (20 mg/10 mL in dimethyl sulfoxide, DMSO) were added. The cultures were further grown at 28 °C, 200 rpm for specific period (0, 1, 3, 5, 7, 14 days). For sterilized control experiments, 7-day cultures were sterilized at 110 °C, 30 min and fenazaquin solution (0.5 mL) was treated as described above. After further incubation at the same condition, the concentrations of fenazaquin were analyzed. Media control was also prepared according to the same procedure without fungal mycelia. All experiments were performed in triplicates.

Effects of metabolic inhibitors

The following metabolic inhibitors were selected, including piperonyl butoxide, ketoconazole, and myclobutanil as cytochrome P450 (CYP) inhibitors, and methimazole as a flavin-dependent mono-oxygenase (FMO) inhibitor. The seed culture of *A. niger* KACC 45093 (0.2 g, fresh weight) was added to a freshly sterilized PDB (200 mL), followed by stock solutions of metabolic inhibitors (500 mg/10 mL DMSO). The inhibitor concentrations were set to 1 mg/200 mL PDB, except myclobutanil (1, 5, and 20 mg/200 mL PDB). After 12 h of pre-incubation at 28 °C, 200 rpm, aliquot (0.5 mL) of fenazaquin solutions (20 mg/10 mL, DMSO) was added. After incubations, the amounts of parent and metabolites were measured. Triplicate cultures were prepared for each treatment.

Extraction of metabolites and instrumental analysis

The cultures, including mycelia and medium, were homogenized with Waring blender (2 min) and filtered. The filter cake was extracted with MeOH (100 mL × 2). The combined extracts were concentrated under reduced pressure. The residue was suspended in saturated NaCl (200 mL). Fenazaquin and its metabolites were extracted with ethyl acetate (EA, 100 mL × 3). The EA extracts were dried over anhydrous Na_2SO_4 and concentrated to

dryness. The residue was re-dissolved in EA (10 mL) and used for gas chromatography–mass spectrometry (GC–MS).

Chemical oxidation of fenazaquin

Fenazaquin (30 mg) was dissolved in dichloromethane (80 mL). A solution of MCPBA (2, 6, and 20 mg/20 mL, dichloromethane) was added in one portion and stirred at 25 °C. Aliquots of reaction mixture was collected at several intervals and analyzed with GC–MS.

Instrumental analyses of fenazaquin and metabolites

Fenazaquin and its metabolites were analyzed with gas chromatograph–mass spectrometer (GC–MS). Analytical conditions for GC–MS were as follows: Shimadzu GC-2010 with GCMS-2010 SE) equipped with Rtx-5MS column (30 m, 0.25 µm film thickness, 0.25 nm i.d.; Restek, USA). Helium was carrier gas at a flow rate of 1 mL/min. The column temperature was programmed as follows: 160 °C (10 min) and raised to 295 °C at a rate of 2.5 °C/min and held for 30 min. The mass spectra of metabolites were obtained in full scan mode.

Results and discussion

Kinetics of fungal and chemical degradation of fenazaquin

Fenazaquin was quite stable in control experiments (Fig. 1, Table 1). However, the concentration of remaining fenazaquin showed an exponential decay with reaction time. In addition, a concentration-dependent dissipation was observed in MCPBA-mediated oxidations. For example, the reaction rate with 200 mg/L MCPBA (approximately 10 molar excess to fenazaquin) was 5 times higher than that of 20 mg/L MCPBA (approximately 1 equivalent). Both results indicated that MCPBA-mediated fenazaquin oxidation followed first-order kinetics. MCPBA is frequently used as an oxidative enzyme mimic in pesticide metabolism [27–29]. Numerous functional groups can be oxidized with MCPBA (e.g., including alcohols, aldehyde, carbamates, and organophosphorus) [30]. MCPBA can catalyze oxidative transformation of ethers into ketones [31]. In addition to MCPBA, several peracids are also used in chemical synthesis and metabolic studies [27, 28]. Extensive studies showed that the reaction products of drugs and pesticides via peracid oxidation are frequently similar to those of CYP-catalyzed metabolism [27–30]. Based on these results, comparative analysis with the fungal metabolism yielded a detailed insight into fenazaquin metabolism.

Fenazaquin was rapidly dissipated under *A. niger* culture. The degradation pattern also showed exponential decay. Biological degradation of xenobiotics often follows first- or pseudo-first-order kinetics [32, 33]. Half-life and rate constants of fenazaquin were 0.6 and 1.13/day, respectively. No degradation was observed in sterilized mycelia and medium control (Fig. 1; Table 1).

Metabolites from chemical and fungal degradation

According to instrumental analyses, the major degradation products of MCPBA oxidation were 4-*t*-butylphenethyl alcohol (II) and 4-hydroxyquinazoline (IV) (Table 2). All metabolites, except 2-(4-(2-hydroxyethyl)-phenyl)-2-methyl-1-propanol (VI), were identified with synthetic standards (Supplementary material). The concentrations of II and IV gradually increased during chemical oxidation and fungal cultures. However, the levels of these

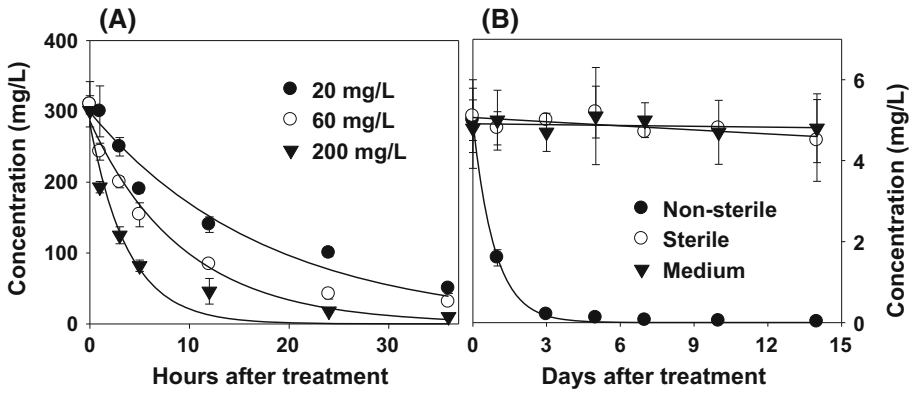

Fig. 1 Degradation of fenazaquin by MCPBA-catalyzed oxidation (**A**) and *A. niger* (**B**). Inserts in panel A indicate the concentrations of MCPBA (symbols for MCPBA concentration: filled circle, 20 mg/L; empty circle, 60 mg/L; filled triangle, 200 mg/L; Inserts in panel B are for non-sterile normal cultures (filled circle), sterilized cultures (empty circle), PDB culture medium without mycelium (filled triangle), respectively

Table 1 First- or pseudo-first-order kinetic parameters of fenazaquin degradation

Classification	Inhibitors	Oxidants (mg/L)	Rate (/day)	Half-lives (day)	$R^{2,a}$
Chemical oxidation by MCPBA	–	20	0.06	0.5	0.9591
		60	0.11	0.3	0.9693
		200	0.26	0.1	0.9622
Biodegradation	Non-sterile	–	1.13	0.6	0.9912
	Sterile	–	ND[b]	–	–
	Medium	–	ND	–	–
Biodegradation inhibitors[c]	MTZ[d]	–	0.44	1.6	0.9962
	PBO	–	0.01	58.7	0.9407
	KCZ	–	0.58	1.2	0.9107
	MCN	–	0.42	1.7	0.9415
	MCN[e]	–	0.38	1.8	0.9696
	MCN[e]	–	0.16	4.3	0.9815

[a]Regression coefficient

[b]No degradation (ND)

[c]Inhibitor concentration (5 mg/L)

[d]*MTZ* methimazole, *PBO* piperonyl butoxide, *KCZ* ketoconazole, *MCN* myclobutanil

[e]Concentration of myclobutanil: 25 and 100 mg/L

Table 2 GC–MS retention times and mass spectral data of fenazaquin and metabolites derived from MCPBA oxidation and cultures of *A. niger*

ID	Name	Retention time (min)	Molecular and fragment ions (% relative abundance)
I	Fenazaquin	45.02	306 (M^+, 1), 207 (1), 160 (47), 145 (100), 131 (10), 117 (29)
II	4-*t*-Butylphenethyl alcohol	23.75	178 (M^+, 23), 163 (100), 147 (21), 132 (19), 117 (33), 105 (25), 91 (25)
III	4-*t*-Butylphenylacetic acid	27.24	192 (M^+, 22), 177 (100), 149 (19), 131 (50), 117 (22), 91 (25), 77 (9)
IV	4-Hydroxyquinazoline	30.33	146 (M^+, 100), 118 (32), 91 (24), 64 (23)
V	2,4-Quinazolinedione	34.18	162 (M^+, 82), 133 (7), 119 (100), 92 (75), 64 (25)
VI	2-(4-(2-hydroxyethyl)-phenyl)-2-methyl-1-propanol	30.75	194 (M^+, 1), 176 (2), 163 (100), 145 (21), 133 (18), 117 (32), 105 (25), 91 (21), 77 (8)

metabolites decreased at the end of the experiments (Fig. 2). One of the most notable differences between chemical and biological oxidations was detected with the metabolites 4-*t*-butylacetic acid (III) and 2,4-quinazolinedione (V) in MCPBA oxidation. Because of its strong oxidative activity, MCPBA is commonly used for the

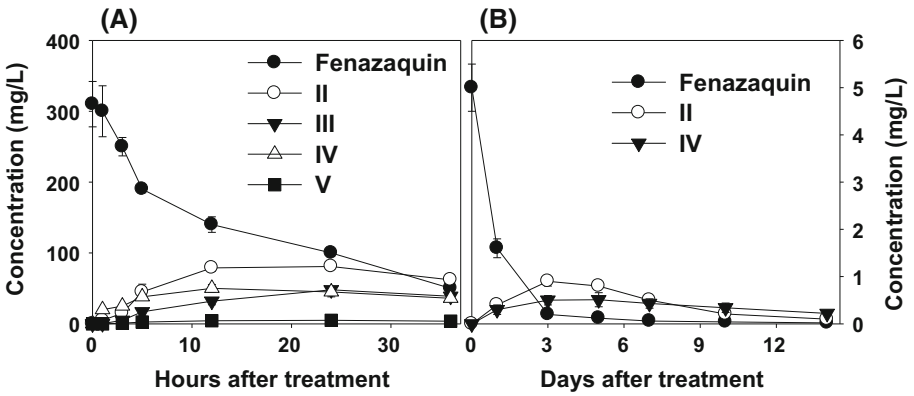

Fig. 2 Concentrations of fenazaquin metabolites during MCPBA-mediated oxidation (**A**) and cultures of *Aspergillus niger* (**B**). Concentration of MCPBA, 60 mg/L. Metabolites II–V are listed in Table 2

oxidation of alcohols to aldehyde or carboxylic acids. Heterocycles are also susceptible to oxidation with the same reagent [27, 29, 30]. Recent studies showed that the same oxidant can oxidize cycloalkyl methyl ether to cyclic ketones [31]. Based on these findings, II and IV may be further oxidized to III and V by MCPBA. Instrumental analysis of *A. niger* cultures yielded II and IV as the major metabolites, with concentrations accounting for 30–50% of fenazaquin transformation. The levels of III and V in fungal culture were negligible. The metabolite VI was tentatively identified as 2-(4-(2-hydroxyethyl)-phenyl)-2-methyl-1-propanol, derived from the oxidation of 4-*t*-butyl group of II or via cleavage of the ether bond cleavage in undetected metabolite(s). Notably, fenazaquin metabolites containing oxidized 4-*t*-butyl group (alcohol and acid) were reported in rats and a few plants [6]. In a photochemical degradation experiment, preferential oxidation of *t*-butyl group over alcohols was observed [34]. Previous study with synthetic flavonoids showed that *A. niger* can rapidly oxidize *t*-butyl group to alcohols and acids by cytochrome P450 [22]. These results indicated that metabolite VI may be originated from metabolite II or fenazaquin alcohol. However, previous studies showed that II and IV were the major metabolites occurring in most biological systems [6, 34, 35]. Accordingly, it is plausible that metabolic pathways of fenazaquin in *A. niger* resemble those of animal and plants.

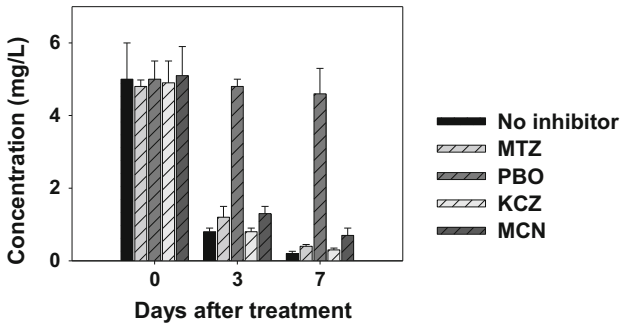

Fig. 3 Effects of metabolic inhibitors on the degradation of fenazaquin. Names of inhibitors are abbreviated as follows: *MTZ* methimazole; *PBO* piperonyl butoxide; *KCZ* ketoconazole; *MCN* myclobutanil. Inhibitor concentrations were 5 mg/L

Effects of inhibitors on fungal metabolism of fenazaquin

Xenobiotics in biological system are subjected to metabolic transformation. Such structural modification of pesticides can result in potentiation or reduction of bioactivity [36, 37]. The binary or ternary mixture of pesticides frequently showed a synergistic effect of acetylcholine esterase inhibition [37]. Similar results were also found in drugs. For example, several antifungal agents (e.g., ketoconazole) are strong inhibitors of drug metabolizing CYP isoforms [38]. Co-application of these fungicides and other drugs reduces the metabolic clearance of drugs, which results in enhanced efficacy. Piperonyl butoxide (PBO) is an important synergistic agent included in many pesticide formulations. It is an established and irreversible inhibitor of CYPs [39]. In this study, three CYP inhibitors were tested for their effects on fenazaquin metabolism. PBO strongly inhibited fenazaquin metabolism, while azole antifungal reagents (ketoconazole and myclobutanil) showed a limited inhibition (Figs. 3, 4; Table 1). The degradation of fenazaquin showed a concentration-dependent reduction by myclobutanil (Table 1). For example, the rate constant at the highest concentration of myclobutanil was one-third of the value observed at the lowest treatment level (0.16 and 0.42/day, respectively). However, these effects may result from the fungi-toxic effects of myclobutanil (Fig. 4). Accordingly, it can be concluded that certain CYPs that are susceptible to PBO-mediated inhibition catalyze fenazaquin metabolism. Flavin-dependent mono-oxygenase (FMO) is another common mediator of xenobiotic metabolism [40]. Until now, no FMO inhibitors have been registered as pesticide synergists. However, methimazole is frequently used as FMO inhibitor in

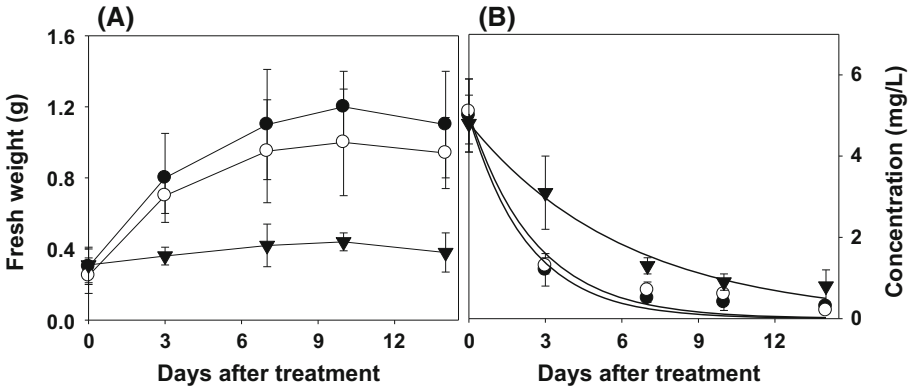

Fig. 4 Fresh weight of mycelia (**A**) and concentration of fenazaquin (**B**) in myclobutanil-treated cultures. Symbols of myclobutanil concentrations are as follows: filled circle, 1 mg/200 mL; empty circle, 5 mg/200 mL; filled triangle, 20 mg/200 mL

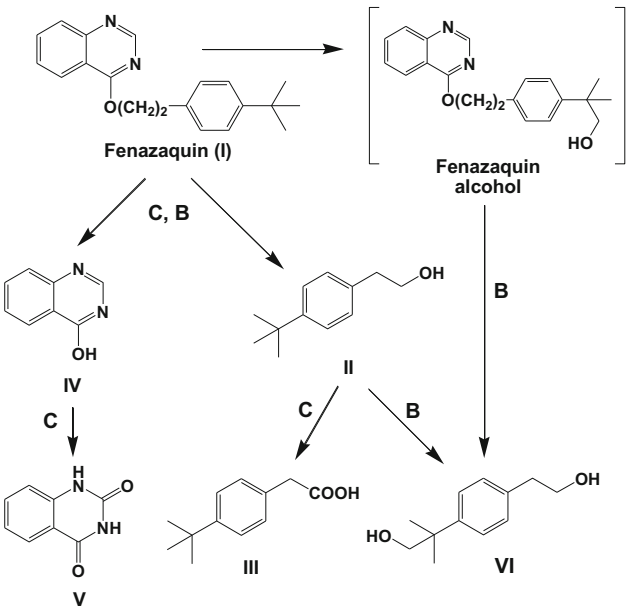

Fig. 5 Degradation pathways of fenazaquin by MCPBA and *A. niger*. Roman numerals indicate metabolite IDs for GC–MS. Bold-faced C and B indicate chemical and biological metabolism. Metabolites in the bracket are reported in the literature

metabolic studies of drugs and pesticides [40, 41]. Methimazole slightly reduced the transformation rate of fenazaquin (Fig. 3; Table 1). The finding indicates that the contribution of FMO is limited in fenazaquin metabolism.

In summary, the fungal metabolic pathway of fenazaquin resembles that of animal and plants (Fig. 5). PBO-selective inhibition indicates that CYPs may be the major enzymes in fenazaquin metabolism. These findings showed that PBO or related synergists enhance the efficacy of pesticides, which in turn reduces the pesticide application via PBO combination. Environmental safety can also be achieved via efficient use of synergists.

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References

1. Solomon MG, Fitzgerald JD, Ridout MS (1993) Fenazaquin, a selective acaricide for use in IPM in apple in the UK. Crop Prot 12:255–258
2. Wood E, Latli B, Casida JE (2003) Fenazaquin acaricide specific binding sites in NADH: ubiquinone oxidoreductase and apparently the ATP synthase stalk. Pestic Biochem Physiol 54:135–145
3. Hollingworth R, Ahmmadsahib K, Gedelhak G, McLaughlin J (1994) New inhibitors of complex I of the mitochondrial electron transport chain with activity as pesticides. Biochem Soc Trans (Lond.) 22:230–233
4. Coscolla C, Yusa V, Beser MI, Pastor A (2009) Multi-residue analysis of 30 currently used pesticides in fine airborne particulate matter (PM 2.5) by microwave-assisted extraction and liquid chromatography-tandem mass spectrometry. J Chromatogr A 1216:8817–8827
5. Tewary DK, Kumar V, Shanker A (2004) Leaching of pesticides in herbal decoction. Chem Health Saf 11:25–29
6. Roberts TR, Hutson DH, Lee PW, Nicholls PH, Plimmer JR (1999) Metabolic pathways of agrochemicals, part 2: insecticides and fungicides. R Society of Chemistry, London, pp 747–751
7. Bhattacharyya J, Pramanik SK, Banerjee H, Bhattacharyya A (2010) Effect of pH on the fate of fenazaquin in soil and water under laboratory-simulated condition. Toxicol Environ Chem 92:1083–1092
8. Kah M, Beulke S, Brown CD (2007) Factors influencing degradation of pesticides in soil. J Agric Food Chem 55:4487–4492
9. Arbeli Z, Fuentes CL (2007) Accelerated biodegradation of pesticides: an overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. Crop Prot 26:1733–1746
10. Maqbool Z, Hussain S, Imran M, Mahmood F, Shahzad T, Ahmed Z, Azeem F, Muzammil S (2016) Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review. Environ Sci Pollut Res Int 23:16904–16925
11. Kamei I, Takagi K, Kondo R (2011) Degradation of endosulfan and endosulfan sulfate by white-rot fungus *Trametes hirsuta*. J Wood Sci 57:317–322
12. Purnomo AS, Putra SR, Shimizu K, Kondo R (2014) Biodegradation of heptachlor and heptachlor epoxide-contaminated soils by white-rot fungal inocula. Environ Sci Pollut Res 21:11305–11312
13. Singh BK, Kuhad RC (1999) Biodegradation of lindane (γ-hexachlorocyclohexane) by the white rot fungus *Trametes hirsutus*. Lett Appl Microbiol 28:238–241
14. Badawi N, Rønhede S, Olsson S, Kragelund BB, Johnsen AH, Jacobsen OS, Aamand J (2009) Metabolites of the phenylurea herbicides chlorotoluron, diuron, isoproturon and linuron produced by the soil fungus *Mortierella* sp. Environ Pollut 157:2806–2812
15. Fang H, Xiang YQ, Hao YJ, Chu XQ, Pan XD, Yu JQ, Yu YL (2008) Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi. Int Biodeterior Biodegr 61:294–303
16. Hangler M, Jensen B, Rønhede S, Sørensen SR (2007) Inducible hydroxylation and demethylation of the herbicide isoproturon by *Cunninghamella elegans*. FEMS Microbiol Lett 268:254–260
17. Pinto AP, Serrano C, Pires T, Mestrinho E, Dias L, Teixeira DM, Caldeira AT (2012) Degradation of terbuthylazine difenoconazole and pendimethalin pesticides by selected fungi cultures. Sci Total Environ 435:402–410
18. Ueyama I, Araki Y, Kurogochi S, Yamaguchi I (1993) Metabolism of the phenylurea fungicide, pencycuron, in sensitive and tolerant strains of *Rhizoctonia solani*. J Pestic Sci 18:109–117
19. Kim YH, Ahn JY, Moon SH, Lee J (2005) Biodegradation and detoxification of organophosphate insecticide, malathion by *Fusarium oxysporum* f. sp. *pisi* cutinase. Chemosphere 60:1349–1355
20. Salama AKM (1998) Metabolism of carbofuran by *Aspergillus niger* and *Fusarium graminearum*. J Environ Sci Health B 33:253–266
21. Liang WQ, Wang ZY, Li H, Wu PC, Hu JM, Luo N, Cao LX, Liu YH (2005) Purification and characterization of a novel pyrethroid hydrolase from *Aspergillus niger* ZD11. J Agric Food Chem 53:7415–7420
22. Lim DS, Lim DH, Lee JH, Oh ET, Keum YS (2017) Structure-oxidative metabolism relationships of substituted flavones by *Aspergillus niger*. J Agric Food Chem 65:3056–3064

23. Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, Batzoglou S, Lee SI, Baştürkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Scazzocchio C, Farman M, Butler J, Purcell S, Harris S, Braus GH, Draht O, Busch S, D'Enfert C, Bouchier C, Goldman GH, Bell-Pedersen D, Griffiths-Jones S, Doonan JH, Yu J, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Peñalva MA, Oakley BR, Momany M, Tanaka T, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes M, Paoletti M, Fischer R, Miller B, Dyer P, Sachs MS, Osmani SA, Birren BW (2005) Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438:1105–1115
24. Sun J, Lu X, Rinas U, Zeng AP (2007) Metabolic peculiarities of *Aspergillus niger* disclosed by comparative metabolic genomics. Genome Biol 8:R182
25. Ffrench-Constant RH (2013) The molecular genetics of insecticide resistance. Genetics 194:807–815
26. Szymanska E, Frydenvang K, Contreras-Sanz A, Pickering DS, Frola E, Serafimoska Z, Nielsen B, Kastrup JS, Johansen TN (2011) A new phenylalanine derivative acts as an antagonist at the AMPA receptor GluA2 and introduces partial domain closure: synthesis, resolution, pharmacology, and crystal structure. J Med Chem 54:7289–7298
27. Casida JE, Ruzo LO (1986) Reactive intermediates in pesticide metabolism: peracid oxidations as possible biomimetic models. Xenobiotica 16:1003–1015
28. Keserü GM (1998) Cytochrome P450 catalysed insecticide metabolism: chemical and theoretical models. Sci Progr 81:245–272
29. Segall Y (2011) Biomimetic chemistry as a useful tool for studying reactive metabolites of pesticides. J Agric Food Chem 59:2845–2856
30. Hussain H, Al-Harrasi A, Green IR, Ahmed I, Abbasa G, Rehmana NU (2014) meta-Chloroperbenzoic acid (mCPBA): a versatile reagent in organic synthesis. RSC Adv 4:12882–12917
31. Kamijo S, Matsumura S, Inoue M (2010) CCl_3CN: a crucial prometer of mCPBA-mediated direct ether oxidation. Org Lett 12:4195–4197
32. Chen J, Wong MH, Wong YS, Tam NF (2008) Multi-factors on biodegradation kinetics of polycyclic aromatic hydrocarbons (PAHs) by *Sphingomonas* sp. a bacterial strain isolated from mangrove sediment. Mar Pollut Bull 57:695–702
33. Zeng F, Cui K, Li X, Fu J, Sheng G (2004) Biodegradation kinetics of phthalate esters by *Pseudomonas fluoresences* FS1. Process Biochem 39:1125–1129
34. Bhattacharyya J, Banerjee H, Bhattacharyya A (2003) Photodecomposition of an acaricide, fenazaquin, in aqueous alcoholic solution. J Agric Food Chem 51:4013–4016
35. Bhattacharyya J, Banerjee H, Das SP, Bhattacharyya A (2005) Metabolism of fenazaquin, an acaricide in tea plant. Bull Environ Contam Toxicol 75:569–573
36. Cedergreen N (2014) Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS ONE 9:e96580. https://doi.org/10.1371/journal.pone.0096580
37. Gressel J (1993) Synergizing pesticides to reduce use rates. In: Duke SO, Menn JJ, Plimmer JR (eds) Pest control with enhanced environmental safety. ACS symposium series, vol 524, American Chemical Society, Washington, pp 48-61
38. Venkatakrishnan K, von Moltke LL, Greenblatt DJ (2000) Effects of the antifungal agents on oxidative drug metabolism. Clin Pharmacokinet 38:111–180
39. Farnham AW (1999) The mode of action of piperonyl butoxide with reference to studying pesticide resistance. In: Jones DG (ed) Piperonyl butoxide the insecticide synergist. Academic Press, San Diego, pp 199–213
40. Phillips IR, Shephard EA (2017) Drug metabolism by flavin-containing monooxygenases of human and mouse. Expert Opin Drug Metab Toxicol 13:167–181
41. Hainzl D, Cole LM, Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem Res Toxicol 11:1529–1535