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REVIEW ARTICLE

Toxicity of *Strobilurins fungicides*: A comprehensive review

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KEYWORDS

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ABSTRACT: Fungicides are being extensively used in the field of agriculture to increase production and reduce fungal infection. Strobilurins have emerged as one of the broadly used fungicides worldwide because of their less toxicity and highly efficient fungicidal activity. It is widely used against powdery mildew, white mold, rot, downy mildew, rust, and rice blast diseases in different crops like soybeans, rice, cereals, vegetables, and fruit trees, etc. Humans can get exposed to strobilurins through fruits or vegetables or water and dermal routes during spraying. During the past few years, strobilurin fungicides have been reported to exert an adverse impact on a variety of non-target organisms, including human beings, due to their large-scale use. To review the experimental and epidemiologic data available showing the association between exposure to strobilurins and health effects. PubMed, Web of Science, Google Scholar etc. were searched for published studies on various Strobilurin fungicides. Based on the review, it was concluded that Strobilurins exert a toxicological impact on aquatic and terrestrial organisms via immunomodulation, cell apoptosis, endocrine disruption, oxidative stress, genotoxicity, etc. However its toxic effects are least reported on mammalian species, but excessive use of Strobilurins during pre and post-harvesting activities can lead to its accumulation in the natural environment that can cause an adverse impact on mammals as well. Therefore, to find out the toxic effects of Strobilurins, more studies should be conducted.

INTRODUCTION

Pesticides are chemical substances commonly used to protect crops from insects, pests and plant disease vectors and foods during storage from rats, mice, insects or various biological contaminants. Pesticides are also used to control the spread of pests that can harm livestock and humans. Pesticides include insecticides, herbicides, rodenticides and fungicides which are used to kill or control insects, weeds, rodents and fungi respectively [1].

Fungal infections or diseases are a major threat in the field of agriculture. To protect crops and animals from fungal infection and secure production rates, fungicides are extensively used [2]. Strobilurins have emerged as a new class of fungicides, which are contact fungicides and can be used as a protectant, curative, and translaminar fungicides. These were isolated from mushrooms (Basidiomycetes) at first. Strobilurin-A, obtained from the mushroom *Strobilurus tenacellus, was* the foremost natural strobilurin compound [3]. In 1996, strobilurin fungicide (azoxystrobin) was patented and launched in the German market. Currently, ten major strobilurin fungicides are available in the market, which accounts for 23-25% of worldwide fungicide sales [4]. Picoxystrobin, azoxystrobin, kresoxim - methyl, orysastrobin, dimoxystrobin, pyraclostrobin, fluoxastrobin and trifloxystrobin are major *strobilurins fungicides*. The major structural characteristic of Strobilurin fungicides is the presence of toxiphoric (E)-β-methoxyacrylate group [5]. Strobilurins are known to act as QoIs (Quinone outside Inhibitors) because they bind to the quinol oxidation site (Qo) of cytochrome b and obstruct electron transport between cytochrome b and cytochrome c1. They also hinder the production of

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nicotinamide adenine dinucleotide (NADH) and the mitochondrial membrane protein ATP [6, 7].

Strobilurin fungicides are widely used to manage infections such as white mold, rot, early and late leaf spot, rust and rice blast in agriculture [4]. Due to increased usage of strobilurins, the residues of strobilurin may enter aquatic ecosystems via the surface and groundwater [8]. Humans can be exposed to strobilurins through the remains of pesticides available in the air, soil, or water [9]. Strobilurins residues persist in the air, water and soil atmosphere after spraying in the field [10]. Generally, strobilurin compounds simply degrade through biological or chemical processes in plants, animals, soil and water. After reaching the ground, it undergoes chemo or biotransformation and may interact with organic or mineral constituents [11-13). Strobilurins products are also used to manage mold and mildew in the pulp and manufacturing of paper, cooling towers and heat exchangers. Strobilurin fungicides present in wallboard can also migrate to the indoor environment and may be present in house dust which can lead to the potential exposure of humans to these chemicals indoors [14].

Strobilurins are considered low-risk fungicides as they are derived from natural products and degrade rapidly in the environment. Strobilurins can be arranged as pyraoxystrobin, pyraclostrobin ≈ trifloxystrobin, picoxystrobin, kresoxim-methyl fluoxastrobin and azoxystrobin in decreasing order of toxicity [15]. Its widespread and long-term use in agriculture and industries has raised serious public health concerns. Few studies have reported increased concentrations of strobilurins above the acceptable regulatory concentration in ecosystems. Soil and water bodies are getting contaminated and their biomagnification is rising which may lead to ecosystem imbalance and food-web disruption. In turn, it may cause adverse effects on soil organisms, aquatic organisms, and mammals [16, 17].

In the present review, various *in-vivo* and *in-vitro* studies conducted on different invertebrate and vertebrate animal models related to the toxic effects of strobilurins have been compiled. Various search engines like Pubmed, Science direct, Web of Science, Scopus etc, were used to search related articles till May, 2022. Following terms or keywords are used for searching relevant papers strobilurin, toxicity, reproduction, developmental toxicity, hepatotoxicity, renal toxicity and cytotoxicity.

Table1. *In vivo* toxicity of Strobilurins in Invertebrate and Vertebrate animal model

S No.	Animal model	Fungicide and Dose	Duration	Observation	Ref.
			INVERTEBRATES		
1	Earthworm (*Eiseniafetida*)	Azoxystrobin (0, 0.1, 1.0 and 2.5 mg kg^{-1})	7,14, 21,28, 42 and 56 days	Azoxystrobin exposure caused the accumulation of reactive oxygen species, enhanced activity of Superoxide dismutase, POD and GST and decreased activity of catalase. MDA content was noted to be amplified after 14-day exposure and DNA damage was also found to be increased as the concentration of azoxystrobin was increased.	[18]
3	*Eiseniafetida*	Fluoxastrobin (0, 0.1, 1.0, and 2.5 mg kg^{-1})	7, 14, 21, and 28 days	Activities of Superoxide dismutase and glutathione S-transferase were noted to be increased in *Eisenia* exposed to Fluoxastrobin at the dose level of 0.1 and 1.0 mg kg^{-1}. Dose and time dependentDNA damage was also observed.	[19]
4	Earthworm (*Enchytraeuscrypticus*)	2 and 5 mg kg^{-1} (AZ) Azoxystrobin	28 days	Azoxystrobin exposure significantly increases the number and total abundance of antibiotic resistance genes (ARGs) in the *E. crypticus*. It also caused an increment in the number of Proteobacteria after exposure to azoxystrobin (2 and 5 mg kg^{-1}) which indicated an unhealthy gut bacterial community.	[20]
5	Crayfish (*Astacusleptodactylus*)	1656 mgL^{-1} and its three sub-doses (828, 414, 207) mg L^{-1}Azoxystrobin	(96 h)	Activities of superoxide dismutase and glutathione peroxidase and the content of malondialdehyde were significantly increased in the liver, pancreas, gill and muscles. Acetylcholinesterase (AChE) and glutathione S-transferase (GST) were increased significantly in hepatopancreas while glutathione reductase (GR) activity and level of reduced glutathione (GSH) decreased significantly. The activity of ATPases was also significantly inhibited in gill and muscle tissues.	[21]

6	Snail (*Lymnaealuteola L)*	Azoxystrobin (0.79 mg L^{-1}), and three sublethal concentrations. (0.079mg L^{-1}), (0.40mgL^{-1}), and (0.53 mg L^{-1}).	96-h	In hemocyte cells, the level of reactive oxygen species and apoptosis was enhanced in a dose- and time-dependent manner. In digestive glands, lipid peroxidation and glutathione S transferase were enhanced while content of glutathione and superoxide dismutase was declined. Severe damage and maximum fragmentation of DNA was noticed.	[22]
7	*Chironomus dilutes*	(Imidacloprid, IMI) LC50values 3.98±1.17µg L^{-1} (Azoxystrobin, AZO) LC50 values 52.9±1.1µg L^{-1}	96-h	Imidacloprid and Azoxystrobin both induced oxidative stress and mitochondrial destruction. Lethality was also noted along with altered expression of cyt b, coi, cox1, cyp4, cyp12m1, cyp9au1, cyp6fv1, cyp315al, gst, Zn/Cu-sod, Mn-sod and cat.	[23]
				FISH	
7	Atlantic salmon (*Salmosalar L.*)	Azoxystrobin (61 mg L^{-1}; 122 mgL^{-1} and 352 mg L^{-1}).	4 days	At High dose treatment, both ion and acid–base regulation were affected, with a significant decrease in Na+ and Cl, and a significant increase in pH, PCO_2, HCO_3 and hematocrit. Catalase, IGFBP1 and MAPK1 transcripts were drastically up-regulated in the liver while in muscle tissue, catalase, transferrin, IGFBP1 and TNFR were up-regulated and CYP1A was downregulated.	[24]
8	Grass carp (*Ctenopharyngodon idella*)	Trifloxystrobin 0.051 (0.046–0.058) mg L^{-1}, Azoxystrobin 0.549 (0.419–0.771) mg L^{-1} and kresoxim-methyl 0.338 (0.284–0.407) mg L^{-1} for juveniles.	48 h	Exposure to all three fungicides i.etrifloxystrobin, azoxystrobin and kresoxim-methyl caused an increase in the activity of catalase and peroxidase while decline in the activity of superoxide dismutase, Three growths related genes i.e. IGF-1, IGF-2 and GHR and two energy related genes CCK and PYY revealed significant inhibition in their expression.	[25]
10	Zebrafish (*Daniorerio*)	2, 20 and 200 µgL^{-1}Azoxystrobin	21 days	At 200 µgL^{-1}, reduced 17β-estradiol, vitellogenin, and gonadosomatic index, increased testosterone concentration, histological alterations in the ovaries and livers along with significant down-regulation of lhb, cyp19b, lhr, cyp19a, vtg1 and vtg2, and up-regulation of cyp17, hsd3b and hsd17b was noted in female zebrafish. In male zebrafish, concentration of 17β-estradiol and vitellogenin was found to be increased while testosterone concentration and gonadosomatic index were reduced after exposure to 20 and 200 µgL^{-1}azoxystrobin. Significant up-regulation of cyp19b, cyp11a, cyp17, cyp19a, hsd3b and hsd17b, vtg1 and vtg2 accompanied by histological alterations in the testes and livers were also observed. However, at 2 µgL^{-1}azoxystrobin dose level, significant up-regulation of cyp11a, hsd3b, cyp19a, vtg1 and vtg2 was observed in male Zebrafish.	[26]
11	Zebrafish (*Daniorerio*)	1, 10, and 100µg L^{-1}Azoxystrobin	7, 14, 21, and 28 days.	In male zebrafish, the Superoxide dismutase activity was significantly decreased after day 21. A significant amplification in Catalase (CAT) and glutathione- S-transferase (GST) activity in female zebrafish at 10 and 100µgL^{-1}Azoxystrobin was noticed after 21 days of exposure. Lipid peroxidation and DNA damage were also found to be increased in a concentration-dependent manner.	[27]
12	Zebrafish (*Daniorerio*)	Fluoxastrobin (0.001, 0.01, and 0.1 mg L^{-1})	7, 14, 21, and 28 days	At 0.1 mgL^{-1} dose level, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) were inhibited. Lipid peroxidation and DNA damage were stimulated by ROS.	[28]
13	Larval and adult Zebrafish (*Daniorerio*)	0.01, 0.05 and 0.20 mg L^{-1}Azoxystrobin	8 days	Azoxystrobin (0.05 mgL^{-1}) exposure caused an increment in the concentration of reactive oxygen species, MDA as well as enhanced expression of mRNA levels of immune-related genes (il1b, il8 and cxcl-c1c) in larval and adult Zebrafish. The activity of mitochondrial complex III, ATP concentration and cytb was found to be decreased while sod1, sod2, cat, il1b, il8 and cxcl-c1c, ROS and MDA concentration was increased in larval and adult Zebrafish at 0.20 mgL^{-1}.	[29]
14	Zebrafish (*Daniorerio*)	In Embryo (Azoxystrobin) 0,150,300, 500, 1000,1500, and 2000mg L^{-1}) or (Picoxystrobin) 0, 15, 25, 50, 100, 200, and 400 mg L^{-1} In larva (Azoxystrobin) 0, 0.25, 2.5, 25, and 250 mg L^{-1} and (Picoxystrobin) 0, 0.02, 0.2, 2, and 20 mg L^{-1}	24 to 144 h	The embryo mortality was significantly enhanced in Zebrafish after 24 h exposure to Azoxystrobin at more than 1000 mgL^{-1} and after 48 h exposure to Picoxystrobin at more than 100 mgL^{-1}. The hatching of eggs and embryo development was delayed. Significant embryo malformation effects, including tail deformities, pericardial edema, abnormal yolk sac, and spinal curvature were noted at higher level exposure to both azoxystrobin and picoxystrobin. Activities of catalase, peroxidase, carboxylesterase, glutathione S-transferase, and malondialdehyde content were increased in azoxystrobin, and picoxystrobin-treated zebrafish larvae.	[30]
15	Zebrafish (*Daniorerio*)	2, 20 and 200 µgL^{-1} (Azoxystrobin)	21 days	Increased mortality, higher malformation rate, decreased hatching rate, shorter total body length as well as up-regulated cyp19b, vtg1, vtg2, p53, casp3 and casp9 mRNA and down-regulated sod1 and sod2 mRNA in F1 embryos. At 200 µgL^{-1}, increase expression level of sod1, sod2, cat, p53, and casp9 was also noted.	[31]

16	Zebrafish (*Daniorerio*)	0.2, 2.0, and 20.0 μgL^{-1} (Azoxystrobin	120-day post fertilization	Azoxystrobin exposure induced retarded gonadal development, interrupted synthesis of sex hormone and vitellogenin at 20.0 μgL^{-1} in females and altered mRNA levels of transcripts in males. Reproductive ability was found deteriorated. Developmental defects were also noted in F1 embryos from parents exposed to 20.0 μgL^{-1}azoxystrobin.	[32]
17	Zebrafish (*Daniorerio)*	Trifloxystrobin (8, 40 and 200 mgL^{-1}), kresoxim-methyl (16, 80 and 400 mg L^{-1}), Azoxystrobin (20, 100 and 500 mg L^{-1})and Pyraclostrobin (at 1, 5, and 25 mg L^{-1}.	72 h-LC50	Among all four, Pyraclostrobin (PY) was reported to show the highest acute toxicity to embryos, larvae, juveniles, and adults with 96 h-LC50 at 0.048 mg L^{-1}, 0.029 mg L^{-1}, 0.039 mg L^{-1}, 0.031 mg L^{-1} respectively. Activities of catalase, and superoxide dismutase were reduced while the level of H_2O_2, malondialdehyde, and reactive oxygen species was elevated. Reduction in mitochondrial membrane potential (MMP) was also noted by exposure to Strobilurins.	[33]
18	Zebrafish (*Danio rerio*)	Azoxystrobin100 µg L^{-1}	120 h	Catalase and superoxide dismutase activity reduced while Lipid peroxidation significantly increased. Expression of sod1, sod2, and gpx1b up-regulated, and gpx1a expression down-regulated	[34]
19	Zebrafish (*Danio rerio*)	Azoxystrobin (500, 1000, 2000 and 4000 ppm) and Pyraclostrobin (50, 100, 250, and 500 ppm)	48, and 96 h	Zebrafish exposed to Pyraclostrobin (PY) showed high mortality and malformation in comparison to Azoxystrobin. Delayed body length growth, dramatic changes in shape and size of the heart, and pericardial edema was also noticed in Pyraclostrobin-treated zebrafish embryos.	[35]
20	Zebrafish (*Daniorerio*)	Azoxystrobin and Pyraclostrobin at 0.1, 10, 100 mg L^{-1}	4 hpf to 48 hpf	A significant reduction was observed in both basal and maximal respiration at 48 hpf in treated Zebrafish. Standard body length significantly declined with exposure to pyraclostrobin and azoxystrobin at 5 dpf.	[36]
21	Zebrafish embryo/larvae	Pyraclostrobin (0.0125, 0.125, 1.25, 2.50 µM), Trifloxystrobin (0.25, 0.50, 1.00 and 2.00 µM), or Azoxystrobin (1.00, 5.00, 10.0 and 20.0 µM).	48-h	Exposure to Pyraclostrobin and Azoxystrobin induced hyperactivity whereas trifloxystrobin resulted in hypoactivity in Zebrafish larvae. Pyraclostrobin exposure impaired the inflation of the swim bladder and caused down-regulation of annexin A5 (anxa5) and up-regulation of pre-B-cell leukemia homeobox 1a (pbx1a) mRNA levels. Results obtained from Molecular docking revealed that Azoxystrobin showed more effective interaction with iodotyrosinedeiodinase, prolactin receptor, the antagonistic conformation of thyroid hormone receptor β, and glucocorticoid receptor (GR) as compared to pyraclostrobin and trifloxystrobin.	[37]
22	Cichlid *(Australoherosfacetus)*	0, 0.05, 0.5, 5 and 50 μgL^{-1} (Azoxystrobin)	48 h.	At 50 μgL^{-1}Azoxystrobin induced genotoxicity in both juvenile and adult fish. The inhibited activity of superoxide dismutase (SOD) and increased activity of Glutathione- S- transferases (GST) was noted in the liver and gills of juvenile fish after exposure to Azoxystrobin. In adult fish, increased catalase (CAT) activity and MDA content in the liver along with alteration in H_2O_2 content in gills was also recorded.	[38]
23	Zebrafish (*Daniorerio*)	(100.85µg L^{-1} (Azoxystrobin), 78.35µg L^{-1} (Kresoxim-methyl), 96.95µg L^{-1} (Pyraclostrobin) and 102.10µg L^{-1} (Trifloxystrobin).	24, 48, 72 and 96	Pyraclostrobin and trifloxystrobin caused mitochondrial dysfunction associated with changes in mitochondrial complex III activity and transcripts of oxidative respiration and stress-related genes.	[39]
24	Zebrafish embryo	(1, 10 and 100 μgL^{-1}) Azoxystrobin	96 hpf (h post fertilisation)	At 96 hpf, Azoxystrobin provoked a significant increase in the level of reactive oxygen species at 1 and 100 µg L^{-1} and the activity of superoxide dismutase and AChE activity was also increased at 1 µg L^{-1}. The Catalase and lipid peroxidation showed lesser activity at 1 µg L^{-1} and higher activity at 10 and 100 µg L^{-1}. Incidence of malformations was increased at the highest concentration.	[40]
			AMPHIBIAN		
25	Xenopustropicalis embryos	4 Strobilurins (Pyraclostrobin, Trifloxystrobin, Picoxystrobin and Azoxystrobin), 2 succinate dehydrogenase inhibitors (Isopyrazam and Bixafen), 2 Triazoles (Tebuconazole and myclobutanil), Fludioxonil and Folpet	48 hours	Strobilurins, Succinate dehydrogenase inhibitors, and fludioxonil induced malformations in embryos. Severe malformations were reported in embryos exposed to pyraclostrobin (5.47 mg L^{-1}), trifloxystrobin (37.32 mg L^{-1}), picoxystrobin (51.59 mg L^{-1}), or azoxystrobin (150.60 mg L^{-1}). Bent notochord, hypopigmentation, and enlarged proctodaeum were noticed in embryos exposed to Fludioxonil (0.56 mg L^{-1}). Isopyrazam (1.54 mg L^{-1}) and Bixafen (1.46 mg L^{-1}) treated embryos were also found to be suffering from microcephaly, hypopigmentation, enlarged proctodaeum and narrow fins. However, Folpet and Triazole fungicides showed minor teratogenic effects on embryos.	[41]

26	Xenopustropicalis embryos	Pyraclostrobin (0.5–6 μgL^{-1}), Trifloxystrobin (5–40 μgL^{-1}), Azoxystrobin (10–200$\mu g\ L^{-1}$), Isopyrazam (0.1–2.0 mg L^{-1}), and Bixafen (0.1–2.0 mg L^{-1})	48 hours	Xenopus embryos exposed to mixtures of multiple fungicides revealed a higher incidence of lethality and teratogenicity in comparison to a single fungicide at the corresponding doses. Individual fungicides also showed lethal and teratogenic effects including microcephaly, hypopigmentation, somite segmentation and narrow fin but exposure to mixture of fungicides resulted in additive or synergistic effects.	[42]
				Mammals	
27	Mice	0, 10, 100, or 400 mg kg^{-1} Pyraclostrobin	3 hours	Pyraclostrobin in corn oil displayed adverse health outcomes including loss of body weight, hypothermia and diarrhea at lower doses when added to feed or to aqueous vehicles.	[43]
28	Mice	Azoxystrobin and Iprodione fungicides at concentrations ranging from 0.001 to 100 µM.	24 or 48 h	Azoxystrobin and Iprodione fungicides inhibited the activity of macrophage lysosomal enzyme. Lipopolysaccharide stimulated production of tumor necrosis factor α and nitric oxide by peritoneal macrophages and ConA-stimulated the production of IFNγ by splenocytes was also reported to be inhibited in a dose-dependent manner.	[44]
29	Male Albino rats	(5mL kg^{-1}b.w.)	28 days	Increased ALT, AST, ALP, urea, and creatinine along with decreased glutathione (GSH) and increased malondialdehyde (MDA) were noted. In addition, degeneration of some tubular epithelial cells, hemorrhage in the kidney and severe inflammatory cell infiltration in the liver were also observed.	[45]
30	Rat	(Acetamiprid) (20, 10, and 5 mg kg^{-1} b wt.) (Azoxystrobin) (500, 250, and 125 mg kg^{-1} b wt.)	30 and 60 days	Serum and testis hormone levels of rats decreased with increasing concentrations of AC and AZ. AC was found to accumulate in the liver more than serum and testis, while AZ was distributed between the testis and liver with a low residue in serum.	[46]
31	Mice	I: (0.0002, 0.02, and 2 mg kg^{-1}) Azoxystrobin II: Copulatory plug-positive females were treated with corn oil or Azoxystrobin at a concentration of 2 mg kg^{-1} III: Dams (9–12 wk-old) were treated with corn oil or Azoxystrobin (2 mg kg^{-1})	I: acute II: From E 0.5 to E 14.5 once daily by oral gavage. III: From postnatal day 1 to 12 once daily by oral gavage.	Azoxystrobin accumulated in the cerebral cortex of embryonic and weanling mice of mothers exposed to azoxystrobin indicating that azoxystrobin could be transfer from exposed dams to offspring during the prenatal and postnatal periods via the placenta and breast milk, respectively. The urine concentration of AZ and AZ-acid was also found to be varied in a dose- and time- dependent manner in treated mice. Exposure to azoxystrobin during the lactational period did not alter the body weight or cortex weight of the pups.	[47]

Table 2. *In vitro* toxicity studies of strobilirubin.

So. No.	Cell culture used	Fungicide and dose	Duration	Observation	Ref.
1.	Human peripheral blood lymphocytes cell culture	Signum (2,6 and 25 $lgml^{-1}$), Boscalid (0.5 and 2 $lgml^{-1}$), Pyraclostrobin (0.5, 1.5 and 2.0 $lgml^{-1}$)	44 h	Cytotoxicity was found to be increased in exposed cells with increasing concentrations of. Formation of MN (Micronuclei) in proliferating lymphocytes was statistically increased in cells exposed to signum, boscalid, and pyraclostrobin. Nucleoplasmic bridge formation was also found to be increased in proliferating lymphocytes exposed to signum, and pyraclostrobin. Pyraclostrobin (0.75 $lgmL^{-1}$) increased nuclear bud (NBUDs) frequencies.	[48]
2.	Cultured mouse cortical neurons.	0.1–100 mM — ametoctradin, boscalid, cyazofamid, dimethomorph, fenhexamid, kresoxim-methyl, mepanipyrim, metrafenone, and pyraclostrobin	7 days	Kresoxim-methyl (KR) and pyraclostrobin (PY) were the most neurotoxic compounds. Evaluated fungicides — cyazofamid, fenhexamid, kresoxim-methyl, metrafenone, and pyraclostrobin were able to significantly induce a rapid rise in intracellular calcium and strong depolarization of mitochondrial membrane potential.	[49]
3.	Mammalian and fish cell lines	Azoxystrobin LC50, 96h/IC50, 48h = 0.581. LC50, 96h/IC50, 72h = 0.998.	48 hours, 72 hours	Azoxystrobin revealed time-dependent cytotoxicity. Among six, HepG2 cells were found to be most sensitive against azoxystrobin exposure. Cell proliferation was inhibited in a concentration-dependent manner within 24 hours while the proliferation of other cell lines was also inhibited after 48 hours.	[50]

4.	3T3-L1 cells obtained from Zenbio	Pyraclostrobin (1.0 and 10.0 µM)	10 days	Pyraclostrobin exposure induced accumulation of triglyceride and decline in steady-state ATP, mitochondrial membrane potential, basal mitochondrial respiration, ATP-linked respiration, and spare respiratory capacity. Expression of Glut-4 (glucose transport), Pkm, Pfkl, Pfkm (glycolysis), Cpt-1b (fatty acid oxidation), and Fasn, Acacα, Acacβ (lipogenesis) genes were reduced which indicated disruption in metabolism.	[51]
5.	Primary cortical neurons were isolated from the embryos of C57BL/6J mice at the E15.5 stage	Azoxystrobin at 30 and 10 µM,	Acute (24h)and chronic (7 days)	Reduced neuronal viability, neurite outgrowth, and cortical migration process was noted in developing brains.	[52]

Mechanism of action

Strobilurins are known to have highly toxic effects on invertebrate animals, fish, and amphibians but few studies are done in mammals. Strobilurins are known to exert harmful effects by various mechanisms, including deteriorating antioxidant defense systems and enhancing oxidative stress, DNA damage, mitochondrial dysfunction, enzymatic disorders, and apoptosis etc.

Oxidative stress

Strobilurins can provoke the generation of excessive oxygen radicals that can cause enhancement in MDA content, consequently leading to oxidative stress, which may target DNA, cell membrane or other cellular components [53]. Several studies in aquatic organisms indicated that strobilurins exert oxidative stress by interrupting the action or the transcription of major enzymes involved in the antioxidant system [25, 30, 54-57]. Azoxystrobin, and picoxystrobin have been reported to induce a significant decline in the activities of Catalase, Superoxide dismutase, and peroxide or elevation in the reactive oxygen species, and Malondialdehyde concentrations in the liver of larvae and adult zebrafish [29, 30]. A similar increase in reactive oxygen species and MDA contents were noticed in Zebrafish exposed to pyraclostrobin, trifloxystrobin, and picoxystrobin [56, 57] and in grass carp juveniles exposed to trifloxystrobin, azoxystrobin, and kresoxim-methyl [25].

DNA damage or genotoxicity

Strobilurins are known to induce DNA damage, including strand breaks, the removal of nucleotides, and various modifications of the nucleotide bases. It can induce toxic effects by interacting with DNA strands and disrupting their replication and transcription or interacting with the nuclear proteins, mitotic spindles, checkpoints and inhibition of antioxidant defense mechanisms. DNA damage can also be correlated with the accumulation of ROS or mitochondrial dysfunction [58]. Comet assay reflects DNA damage by enhanced olive tail moment (OTM) levels in Zebrafish exposed to Azoxystrobin, pyraclostrobin and fluoxastrobin [56, 28]. Another biomarker of DNA damage, 8-hydroxy-2deoxyguanosine (8-OHDG) was also noted to be increased in Fluoxastrobin revealed fish embryos, which provides another evidence of DNA damage induced by Strobilurins[34].

Strobilurin- induced oxidative stress may also lead to alteration in the expression of genes which in turn can cause genotoxicity. Affected expression of p53 [9] sod1, sod2, gpx1b and gpx1a [34], immune-related genes (cxcl-c1c, ccl, il-1b, il-8, tnfa and il, tnf) [59, 60] in larval and adult fish indicated genotoxicity induced by azoxystrobin, Pyraclostrobin, trifloxystrobin, picoxystrobin. Luz *et al.*, [51] also reported similar genotoxic effects in 3T3-L1 cells exposed to Pyraclostrobin which may lead to interference in metabolism.

Mitochondrial dysfunction

Mitochondria acts as a central site for the Krebs cycle, electron transport system, synthesis of amino acids, biosynthesis of heme, and controlling cellular iron homeostasis. It also plays a significant role in regulating the production of reactive oxygen species [61]. Excessive stimulation of NAD(P)H and the electron transport chain leads to the overproduction of ROS, which can result in oxidative stress in mitochondria [62]. Oxidative stress inside mitochondria may lead to mutations in mitochondrial DNA, disturbances in the mitochondrial respiratory chain, affect Ca^{2+} homeostasis, and alter membrane permeability. Several studies on mammalian or fish cell lines indicated that Strobilurins inhibit mitochondrial respiration by triggering the mitochondrial pathway [63, 49, 64, and 65].

According to Li *et al.*,[39] disturbances in mitochondrial bioenergetics can be correlated with the lesser activity of complex III and reduced cytb mRNA levels which results in diminished electron supply to complex IV and V. Mitochondrial dysfunction in Zebrafish due to exposure to strobilurins has also been supported by Cao *et al.*,[29]. Gene Ontology (GO) analysis done by Jiang *et al.*, [69] indicated that Strobilurins can disturb the component of organelle membrane, which can induce mitochondrial dysfunction, and damage mitochondrial ultrastructure.

Apoptosis

Caspases play a crucial role in the apoptotic pathway. Caspase-9 acts as an initiator caspase, which activates the caspase-3 and eventually causes apoptotic changes in cells [66]. Strobilurins can induce apoptosis by activating caspase 3/ caspase 9 and altering the mitochondrial pathway in Zebrafish larvae [65]. Interrupted mitochondria-dependent pathways can promote the generation of reactive oxygen species and eventually lead to increased calcium levels which can cause either apoptosis or necrosis [67, 68]. Disturbed Ca^{2+} and matrix metalloproteinases (MMP) levels followed by activation of caspase-3 and caspase 9 might lead to irreparable apoptosis in zebrafish larvae [69].

Ca^{2+} transfer between the Endoplasmic reticulum and mitochondria acts as a critical signal in the apoptotic pathway. Tumor-suppressor protein p53 promotes apoptosis through two mechanisms: It controls cell-death programs within the nucleus as a transcription factor or through a Ca^{2+}-dependent mechanism [70]. The p53 protein interacts and causes an alteration in the oxidative state of sarco/ER Ca^{2+}-ATPase (SERCA) pumps leading to an increased Ca^{2+} level. This high Ca^{2+} level causes changes in mitochondrial morphology and depolarization which leads to a decline in ATP synthesis and induces apoptosis [71]. Morphological alterations in mitochondria caused by enhanced Ca^{2+} level result in the opening of Permeability Transition Pores, mitochondrial fragmentation, and cytochrome *c* release, which lead to apoptosis. Ca^{2+}-dependent mechanisms of apoptosis have been supported by Flampouri*et al.*,[64] and Regueiro*et al.*,[49]who reported a rapid rise in the level of calcium ions in cultured human or mouse cells exposed to strobilurins (KM and PY). Zhu *et al.*, [9] also reported increased expression of p53 in fish embryos treated with Trifloxystrobin (TFS) which can induce expression of the proapoptotic gene (Bax) on the mitochondrial membrane, further activation of effector caspase-3 and accelerated apoptotic rate. A significant decrease in mitochondrial membrane potential after trifloxystrobin, kresoxim-methyl, Azoxystrobin, and pyraclostrobin exposure has also been reported in Zebrafish [72, 65, and 69].

Endocrine disruption

Strobilurins are also known to have the potential to cause endocrine disruption. They may act on the hypothalamic-pituitary-gonad (HPG) axis or the hypothalamic-pituitary-thyroid or adrenal axis and results in sex hormone disruption by affecting gene expression [60, 33].

Strobilurins may also interact with nuclear receptors to disrupt the functioning of the endocrine system [73]. The thyroid receptor (TR) is a member of the nuclear receptor superfamily. Crupkin*et al.*,[38] also indicated the

endocrine disruptive potential of strobilurins. Molecular docking studies conducted by Yang *et al.*, [37] indicated that strobilurin fungicides such as Azoxystrobin could also interact with iodotyrosinedeiodinase, prolactin receptor, and antagonistic conformation of thyroid hormone receptor β and glucocorticoid receptor. Zhu *et al.*,[74] suggested that trifloxystrobin may act as a probable endocrine disruptor by regulating the mRNA level of vtg, cyp17, and cyp19a (involved in the sex hormone pathway) and cyp1a genes (involved in aryl hydrocarbon receptor pathways) in medaka (Oryziaslatipes) exposed to different levels of trifloxystrobin (0, 0.1, 1, 10, and 100 μgL^{-1}).

CONCLUSIONS

Based on the review, it can be concluded that Strobilurins have emerged as a class of fungicides extensively used in agricultural fields, which led to increased contamination of the environment. Strobilurin toxicity on aquatic animals and amphibian embryos is well reported so we can assume that the level of strobilurin residues is rising in trophic level due to biomagnification which may result in ecosystem imbalance. Although a few studies are available in mammalian toxicity, it's time to raise concerns to analyze the toxic effects of strobilurins. Therefore more studies should be planned to fill the gaps in current toxicological understandings of the effects of strobilurins in mammal animal models and to develop degradation technologies for these pesticides.

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Conflict of interests

There is no conflict of interest.

REFERENCES

1. Lushchak V.I., Matviishyn T.M., Husak V.V., Storey J.M., Storey K.B., 2018. Pesticide toxicity: a mechanistic approach. Experimental and Clinical Sciences. 17, 1101-1136.

2. Latijnhouwers M., de Wit P.J., Govers F., 2000. Oomycetes and fungi: similar weapons to attack plants. Trends in Microbiology. 11, 462-469.

3. Feng Y., Huang Y., Zhan H., Bhatt P., Chen S., 2020. An Overview of Strobilurin Fungicide Degradation: Current Status and Future Perspective. Frontiers in Microbiology. 12, 11-389.

4. Bartett D.W., Clough J.M., Godfrey C.R.A., Godwin J.R., Hall A.A., Heaney S.P., 2001. Understanding the strobilurin fungicides. Pesticides Outlook. 12, 143-148.

5. Balba H., 2007. Review of strobilurin fungicide chemicals. Journal of Environmental Sciences and Health. 42, 441–451.

6. Hnatova M., Gbelska Y., Obernauerova M., Subikova V., Subik J., 2003. Cross resistance to strobilurin fungicides in mitochondrial and nuclear mutants of Saccharomyces cerevisiae. Folia Microbiologica. 48, 496-500.

7. Isamu Y., Makoto F., 2005. Recent topics on action mechanisms of fungicides. Journal of Pesticides Sciences. 30, 67-74.

8. Cui F., Chai T., Liu X., Wang C., 2017. Toxicity of three strobilurins (kresoxim-methyl, pyraclostrobin, and trifloxystrobin) on Daphnia magna.Environmental Toxicology and Chemistry. 36(1), 182–189.

9. Zhu B., Liu GL., Liu L., Ling F., Wang G.X., 2015a. Assessment of trifloxystrobin uptake kinetics, developmental toxicity and mRNA expression in rare minnow embryos. Chemosphere. 120, 447-455.

10. Rodrigues E. T., Lopes I., Pardal M.A., 2013. Occurrence, fate and effects of azoxystrobin in aquatic ecosystem. Environment International.53, 18-28.

11. Bending G.D., Lincoln S.D., and Edmondson R.N., 2006. Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. Environment Pollution. 139, 279-287.

12. Xiao Y., Chen S., Gao Y., Hu W., Hu M., Zhong G., 2015. Isolation of a novel beta-cypermethrin degrading strain Bacillus subtilis BSF01 and its biodegradation

pathway.Applied Microbiology and Biotechnology. 99, 2849–2859.

13. Yang J., Feng Y., Zhan H., Liu J., Yang F., Zhang K., 2018. Characterization of a pyrethroid-degrading Pseudomonas fulva strain P31 and biochemical degradation pathway of D-phenothrin.Frontiers in Microbiology. 16, 9-1003.

14. Cooper E.M., Rushing R., Hoffman K., 2020. Strobilurin fungicides in house dust: is wallboard a source? Journal of Exposure Science and Environmental Epidemiology. 30(2), 247-252.

15. Wang X., Li X., Wang Y., Qin Y., Yan B., Martyniuk C.J., 2021. A comprehensive review of strobilurin fungicide toxicity in aquatic species: Emphasis on mode of action from the zebrafish model. Environment Pollution. 15, 275-116671.

16. Warming T. P., Mulderij G., Christoffersen K.S., 2009. Clonal variation in physiological response of Daphnia magna to the strobilurin fungicides azosystrobin. Environmental Toxicology and Chemistry. 28, 374-380.

17. Zubrod J.P., Bundschuh M., Arts G., Bruhl C. A., Imfeld G., Knabel A., 2019. Fungicides: an overlooked pesticide class. Environmental Science & Technology. 53, 3347-3365.

18. Han Y., Zhu L., Wang J., Wang J., Xie H., Zhang S., 2014. Integrated assessment of oxidative stress and DNA damage in Earthworms (Eiseniafetida) exposed to azoxystrobin. Ecotoxicology and Environmental Safety.107, 214-219.

19. Zhang C., Zhu L., Wang J., Wang J., Du Z., Li B., Zhou T., Cheng C., Wang Z., 2018. Evaluating subchronic toxicity of fluoxastrobin using earthworms (*Eiseniafetida*). Science of the Total Environment. 15(642), 567-573.

20. Zhang Q., Zhu D., Ding J., Zheng F., Zhou S., Lu T., Zhu Y. G., Qian H., 2019. The fungicide azoxystrobin perturbs the gut microbiota community and enriches antibiotic resistance genes in *Enchytraeus crypticus*. Environment International.131, 10496.

21. Uckuna A.A., Oz O.B., 2021. Evaluation of the acute toxic effect of azoxystrobin on non-target crayfish (Astacus leptodactylus Eschscholtz, 1823) by using oxidative stress enzymes, ATPases and cholinesterase as biomarkers. Drug and Chemical Toxicology. 44(5), 550-557.

22. Ali D., Ibrahim K.E., Hussain S.A., and Abdel-Daim M.M., 2021. Role of ROS generation in acute genotoxicity of azoxystrobin fungicide on freshwater snail Lymnealuteola L. Environmental Science and Pollution Research. 28(5), 5566-5574.

23. Wei F., Wang D., Li H., You J., 2021. Joint toxicity of imidacloprid and azoxystrobin to Chironomusdilutus at organism, cell, and gene levels. Aquatic Toxicology. 233, 105783.

24. Olsvik A.P., Kroglund F., Finstad B., Kristensen T., 2010. Effects of the fungicide azoxystrobin on Atlantic salmon (*Salmosalar* L.) smolt. Ecotoxicology and Environmental Safety. 73(8), 1852-1861.

25. Liu L., Jiang C., Wu Z. Q., Gong Y.X., and Wang G. X., 2013. Toxic effects of three strobilurins (trifloxystrobin, azoxystrobin and kresoxim-methyl) on mRNA expression and antioxidant enzymes in grass carp (*Ctenopharyngodon idella*) juveniles. Ecotoxicology and Environmental Safety. 98, 297-302.

26. Cao F., Zhu L., Li H., Yu S., Wang C., Qiu L., 2016. Reproductive toxicity of azoxystrobin to adult zebrafish (*Daniorerio*). Environmental Pollution. 219, 1109-1121.

27. Han Y., Liu T., Wang J., Zhang C., Zhu L., 2016. Genotoxicity and oxidative stress induced by the fungicides azoxystrobin in zebrafish (*Daniorerio*) Liver. Pesticide Biochemistry and Physiology. 133, 13-19.

28. Zhang C., Zhou T., Wang J., Zhang S., Zhu L., Du Z., Wang J., 2018b. Acute and chronic toxic effects of fluoxastrobin on Zebrafish (*Daniorerio*). Science of the Total Environment. 611, 769-775.

29. Cao F., Wu P., Huang L., Li H., Qian L., Pang S., Qiu L., 2018. Short-term developmental effects and potential mechanism of azoxystrobin in larval and adult zebrafish (*Daniorerio*). Aquatic Toxicology. 198, 129-140.

30. Jia W., Mao L., Zhang L., Zhang Y., Jiang H., 2018. Effects of two strobilurins (azoxystrobin and picoxystrobin) on embryonic development and enzyme activities in juveniles and adult fish livers of zebrafish (*Daniorerio*). Chemosphere. 207, 573-580.

31. Cao F., Li H., Zhao F., Wu P., Qian L., Huang L., Pang S., Martyniuk J.C., Qiu L., 2019a. Parental exposure to azoxystrobin causes developmental effects and disrupts gene expression in F1 embryonic zebrafish (*Daniorerio*). Science of the Total Environment. 646, 595-605.

32. Cao F., Martyniuk C.J., Wu P., Zhao F., Pang S., Wang C., Qiu L., 2019b. Long-Term Exposure to Environmental Concentration of Azoxystrobin Delays Sexual Development and Alters Reproduction in Zebrafish (*Daniorerio*). Environ Sci Technology. 53(3), 1672-1679.

33. Jiang J.H., Ly L., Wu S.G., An X.H., Wang F.D., Liu X.J., Zhao X.P., 2019a. Development toxicity of kresoxim-methyl during Zebrafish (*Daniorerio*) larval development. Chemosphere. 219, 517-525.

34. Zhang Y., Sheedy C., Nilsson D., Goss G.G., 2020. Evaluation of interactive effects of UV light and nano encapsulation on the toxicity of azoxystrobin on Zebrafish. Nanotoxicology. 14(2), 232-249.

35. Kim C., Choe H., Park J., Kim G., Kim K., Jeon J.H., Moon K.J., Kim J.M., Lee E.S., 2020. Molecular mechanisms of developmental toxicities of azoxystrobin and pyraclostrobin toward zebrafish (*Daniorerio*) embryos: Visualization of abnormal development using two transgenic lines. Environmental Pollution. 270, 116087.

36. Kumar N., Willis A., Satbhai K., Ramalingam L., Schmitt C., Moussa N.M., Crago J., 2020. Developmental toxicity in embryo-larval zebrafish (Daniorerio) exposed to strobilurin fungicides (azoxystrobin and pyraclostrobin). Chemosphere. 241, 124980.

37. Yang L., Huang T., Li R., Souders II. C., Rheingold S., Tischuk C., Li N., Zhou B., Martyniuk C.J., 2020. Evaluation and comparison of the mitochondrial and developmental toxicity of three strobilurins in zebrafish embryo/larvae.Environmental Pollution. 270, 116277.

38. Crupkin A.C., Fulvi A.B., Iturburu F.G., Medici S., Mendieta J., Panzeri A.M., Menone M.L., 2021. Evaluation of hematological parameters, oxidative stress and DNA damage in the cichlid elomore *Australoheros facetus* exposed to the fungicide azoxystrobin. Ecotoxicogy and Environment Safety. 207, 111286.

39. Li X. Y., Qin Y.J., Wang Y., Huang T., Zhao Y. H., Wang X. H., Yan B., 2021. Relative comparision of strobilurin fungicides at environmental levels: Focus on mitochondrial function and larval activity in early staged zebrafish (*Daniorerio*). Toxicology. 452, 152706.

40. Vieira R.S.F., Venancio C.A.S., Felix L.M., 2021. Embryonic zebrafish response to a commercial formulation of azoxystrobin at environmental concentrations. Ecotoxicology and Environmental Safety. 211, 111920.

41. Li D., Liu M., Yang Y., Shi H., Zhou J., He D., 2016. Strong lethality and teratogenicity of strobilurins on *Xenopus tropicalis* embryos: Basing on ten agricultural fungicides. Environmental Pollution. 208, 868–874.

42. Wu S., Lei L., Liu M., Song Y., Lu S., Li D., He D., 2018. Single and mixed toxicity of strobilurin and SDHI fungicides to *Xenopus tropicalis* embryos, Ecotoxicology and Environmental Safety. 153, 8–15.

43. Tuttle A.H., Salazar G., Cooper E.M., Stapleton H.M., Zylka M.J., 2019. Choice of vehicle affects Pyraclostrobin toxicity in mice. Chemosphere. 218, 501-506.

44. Naasri S., Helali I., Aouni M., Mastouri M., Harizi H., 2020. N-acetylcysteine reduced the immunotoxicity effects induced *in vitro* by azoxystrobin and iprodione fungicides in mice. Environmental Toxicology. 36(4), 562-571.

45. Ziada R.M., Abdulrhman S.M., Nahas A.A., 2020. Hepato-nephro-toxicity induced by premium fungicide and protective effect of sesame oil in male rats.Egyptian journal of hospital medicine. 81(7), 2445-2450.

46. Halawa E., Ryad L., E1-Shenawy N.S., AL-Eisa R.A., EL-Hak H.N.G., 2021. Evaluation of acetamiprid and azoxystrobin residues and their hormonal distrupting effects on male rats using liquid chromatography-tandem mass spectrometry. PLoS One. 16(12), 0259383.

47. Hu W., Liu CW., Jiménez J.A., McCoy E.S., Hsiao Y.C., Lin W., Engel S.M., Lu K., Zylka M.J., 2022. Detection of Azoxystrobin Fungicide and Metabolite Azoxystrobin-Acid in Pregnant Women and Children,

Estimation of Daily Intake, and Evaluation of Placental and Lactational Transfer in Mice. Environmental Health Perspectives.130(2), 27013.

48. Cayir A., Coskun M., 2014. Micronuclei, Nucleoplasmic Bridges, and Nuclear Buds Induced in Human Lymphocytes by the Fungicide Signum and Its Active Ingredients (Boscalid and Pyraclostrobin). Wiley Periodicals, Inc. Environmental Toxicology. 29(7), 723-32.

49. Regueiro J., Olguin N., Simal-Gandara J., Sunol C., 2015. Toxicity evaluation of new agriculture fungicides in primary cultured cortical neurons. Environmental Research. 140, 37-44.

50. Rodrigues E.T., Pardal M.A., Laize V., Cancela M.L., Oliveira P.J., Serafim T.L., 2015. Cardiomyocyte H9c2 Cells present a valuable alternative to fish lethal testing for azoxystrobin. Environmental Pollution. 206, 619-26.

51. Luz A.L., Kassotis C.D., Stapleton H.M., Meyer J.N., 2018. The high-production volume fungicide pyraclostrobin induces triglyceride accumulation associated with mitochondrial dysfunction, and promotes adipocyte differentiation independent of PPARY activation, in 3T3-L1 cells. Toxicology. 393, 150-159.

52. Kang J., Bishayee K., Huh S.O., 2021. Azoxystrobin Impairs Neuronal Migration and Induces ROS Dependent Apoptosis in Cortical Neurons. International Journal of Molecular Science. 22, 12495.

53. Draper H.H., Hadley M., 1990. Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology. 86, 421–431.

54. Liu J., Chen S., Ding J., Xiao Y., Han H., Zhong G., 2015. Sugarcane bagasse as support for immobilization of Bacillus pumilus HZ-2 and its use in bioremediation of mesotrione-contaminated soils. Applied Microbiology and Biotechnology. 99, 10839–10851.

55. Garanzini D.S., Menone M.L., 2015. Azoxystrobin causes oxidative stress and DNA damage in the aquatic macrophyte *Myriophyllum quitense*. The Bulletin of Environmental Contamination and Toxicology. 94, 146-151.

56. Zhang C., Wang J., Zhang S., Zhu L., Du Z., Wang J., 2017. Acute and subchronic toxicity of pyraclostrobin in zebrafish (*Daniorerio*). Chemosphere. 188, 510-516.

57. Li H., Cao F.J., Zhao F., Yang Y., Teng M.M., Wang C. J., 2018. Developmental toxicity, oxidative stress and immunotoxicity induced by three strobilurins (pyraclostrobin, trifloxystrobin, and picoxystrobin) in zebrafish embryos. Chemosphere. 2018, 207-781.

58. Breda C.N.S., Davanzo G.G., Basso P.J., Saraiva Camara N.O., Moraes-Vieira P.M.M., 2019. Mitochondria as central hub of the immune system. Redox Biology. 26, 101255.

59. Jiang J.H., Shi Y., Yu R.X., Chen L.P., Zhao X.P., 2018. Biological response of zebrafish after short-term exposure to azoxystrobin. Chemosphere. 202, 56-64.

60. Wang Y.H., Dai D.J., Yu Y.J., Yang G. L., Shen W.F., Wang Q., Weng H.B., Zhao X.P., 2018a. Evaluation of joint effects of cyprodinil and kresoxim-methyl on zebrafish (*Daniorerio*). The Journal of Hazardous Materials. 352, 80-91.

61. King N., 2007. Amino acids and the mitochondria in: Schaffer SW, Suleiman MS, eds. Mitochondria: The Dynamic Organelle / Advances in Biochemistry in Health and Disease. New York, NY: Springer. 151 – 166.

62. Murphy M.P., 2009. How mitochondria produce reactive oxygen species. Biochemical Journal. 417, 1-13.

63. Gao A.H., Fu Y.Y., Zhang K.Z., Zhang M., Jiang H.W., Fan L. X., 2014. Azoxystrobin, a mitochondrial complex III Qo site inhibitor, exerts beneficial metabolic effects *in vivo* and *in vitro*. Biochimica Et Biophysica Acta. 1840, 2212-2221

64. Flampouri E., Mayrikou S., Mouzaki-Paxinou A.C., Kintzios S., 2016. Alterations of cellular redox homeostasis in cultured fibroblast-like renal cells upon exposure to low doses of cytochrome bc1 complex inhibitor kresoxim-methyl. Biochemical Pharmacology. 113, 97-109.

65. Shi X.K., Bian X.B., Huang T., Wen B., Zhao L., Mu H.X., Fatima S.,2017. Azoxystrobin Induces Apoptosis of Human Esophageal Squamous Cell Carcinoma KYSE-150 Cells through Triggering of the Mitochondrial Pathway. Frontiers in Pharmacology.8, 277.

66. Elmore S., 2007. Apoptosis: a review of programmed cell death. Toxicologic Pathology. 35, 495-516.

67. Barlett D.W., Clough J.M., Godwin J.R., Hall A.A., Hamer M., Dobrzanski B., 2002. The strobilurin fungicides. Pest Management Science. 58, 649-662.

68. Orrenius S., Zhivotovsky B., Nicotera P., 2003. Regulation of cell death: the calcium apoptosis link. Nature Reviews Molecular Cell Biology. 4, 552-565.

69. Jiang J.H., Wu S.G., Ly L., Liu X.J., Chen L.Z., Zhao X.P., Wang Q., 2019b. Mitochondrial dysfunction, apoptosis and transcriptomic alterations induced by four strobilurins in zebrafish (*Daniorerio*) early life stages. Environmental Pollution. 253, 722-730.

70. Giorgi C., Bonora M., Sorrentino G., Missiroli S., Poletti F., Suski J.M., 2015. p53 at the endoplasmic reticulum regulates apoptosis in a Ca^{2+}-dependent manner. Proceedings of the National AcademyofSciences.112(6), 1779-1784.

71. Pathak T., Trebak M., 2018. Mitochondrial Ca^{2+} signaling. Pharmacology & Therapeutics. 192, 112-123.

72. Hong L., Wang X., Wu J., Cai W., 2009. Mitochondria-initiated apoptosis triggered by oxidative injury plays a role in total parenteral nutrition–associated liver dysfunction in infant rabbit model. Journal of Pediatric Surgery. 44(9), 1712–1718.

73. Huet M.C., 2000. OECD Activity on Endocrine Disrupters Test Guidelines Development. Ecotoxicology. 9, 77-84.

74. Zhu L., Wang H., Liu H., Li W., 2015b. Effect of trifloxystrobin on hatching, survival, and gene expression of endocrine biomarkers in early life stages of medaka (*Oryzias latipes*). Environmental Toxicology. 30(6), 648-655.