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Review Article

Imidacloprid (IMI) toxicity in fishes: A review

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Abstract
Over the last few decades, the extensive application of pesticides has increased agricultural productivity. Neonicotinoids (NEOs), a fourth generation of pesticides that arose after organophosphates, pyrethroids, and carbamates, are frequently utilized worldwide. Neonicotinoids have been shown to leach from soil and end up in groundwater or runoff, which badly affects the health of various animals. Among these, imidacloprid (IMI) was the first viable neonicotinoid. IMI is a colorless crystal having the chemical family chloronicotinyl (Neonicotinoid) with a photolytic half-life of 1.2h in deionized water irradiated to UV rays and 126 min in tap water formulated as confidor insecticide. Its great potency at low dosage, low volatility, and high-water solubility (hydrophilic) with low bioaccumulation, nevertheless badly affects the body organs (liver, kidney, gills, etc.) of exposed organisms. IMI widely poses significant threats to aquatic ecosystems, particularly fish, due to its potential toxicity. Understanding the multi-dimensional impacts of IMI toxicity in fish is vital to formulating mitigating plans and suitable pesticide alternatives to safeguard aquatic environments. This review article discusses the long-term effects of IMI on fishes, including disruptions in developmental processes, biochemical alterations, oxidative stress, behavior and alteration in various enzyme activities. Despite numerous studies on IMI toxicity in fish, there is a lack of a comprehensive review that compares different aspects of its toxicity in different fish species. Therefore, this review aims to bridge that gap in current knowledge about IMI toxicity in various fish species and provides a strong basis for future research to safeguard aquatic ecosystems from its harmful effects. In addition, the practical importance of Integrated Pest Management (IPM) and the protective potential of various antioxidants against pesticide toxicity have also been highlighted.

Keywords: Biochemical, Fish, Imidacloprid, Physiological, Toxicity

INTRODUCTION

Agrochemical use is heavily required for both pest control and enhancing food production to feed millions of people worldwide (Al-Mamun, 2017). Agrochemicals such as pesticides are applied to eliminate pests, including weeds, fungi, insects, and rodents (Warne and Reichelt-Brushett, 2023). In addition to boosting food and fiber production, pesticides reduce the spread of vector-borne diseases and have significant economic benefits (Hayes *et al.*, 2017). Since the green revolution of 1960's, pesticides have been largely responsible for a two-fold increase in worldwide crop production (Wijerathna-Yapa and Pathirana, 2022). Chemical pesticides are thought to maintain nearly one-fifth (20%) of the yearly crop production, which makes them essential for maintaining the world's food demand (Blacquiere *et al*., 2012). Pesticides are broadly categorized based on their target pest as insecticides, termiticides, fungicides, acaricides, bactericides and virucides (Nayak and Solanki, 2021). The main chemical groups of commonly used pesticides are neonicotinoids (Imidacloprid), phenyl-pyrazole (Fipronil), carbamates (Propoxur), organochlorine (DDT), pyrethroids (Allethrin), and organophosphates like Chloropyrifos that are frequently applied in fields (Sabra and Mehana, 2015; Chia *et al*., 2024). The continued consumption of pesticides has detrimental impacts on the environment, including the aquatic ecosystem and human health (Kumar *et al*., 2023).

In addition, pesticides also have severe impacts on aquatic creatures, including fish. Since fish circulate energy from lower to upper trophic levels, pesticides start to cause biomagnifications in fish tissues, leading to decreased survivability and disturbing ecological balance (Almeida *et al*., 2021). Pesticides also exterminate terrestrial and aquatic wildlife by inducing adverse effects on vital organs, leading to chronic disease and an algal bloom in many waterbodies, which pose a growing danger to human health (Al-Mamun, 2017; Amenyogbe *et al*., 2021). Among various pesticides, IMI is a systemic chloronicotinoid that utilized to manage several pests like rice hoppers, aphids, termites, beetles, psyllids, and plant bugs in various crops such as rice, sunflowers, cereals, and vegetables (Abd-Eldaim *et al.,* 2023; Fouad *et al*., 2024a). IMI mainly attacks the central nervous system of insects by blocking nAChR receptors at post-synaptic membrane (Rigosi and Carroll, 2021). Its low volatility and bioaccumulation rate mainly minimizes the inhalation risks and makes it relatively safer for terrestrial ecosystems. However, because of its higher water solubility, IMI quickly pollutes the aquatic environments through fields runoff, posing a significant ecological risk (Fouad *et al*., 2024; Kamboj *et al*., 2024). This review discusses the direct toxicity impacts of IMI on various freshwater fishes' developmental, physiological, behavioral, biochemical and haematological aspects. In addition, LC50 concentration of IMI for various fishes and the concentration of IMI in different water samples is also reviewed (Table 1). However, the main concern has been the potential destructive effects of IMI on fish.

IMIDACLOPRID AND ITS EXPOSURE TO AQUATIC HABITAT

Imidacloprid (IMI), a neonicotinoid insecticide, had been brought to the pesticide industry by Bayer in 1991 (Elbert *et al*., 2008). It was the first well-recognized Neonicotinoid to be marketed and used for household and agricultural protection (Abou-Donia *et al*., 2008). Tomlin (1997) said that it is a transparent crystalline chemical belonging to the Chloronicotinyl (neonicotinoid) molecular family with the formula $C_9H_{10}ClN_5O_2$ (Fig. 1). It is one of the most valuable pesticides because of its high water solubility (hydrophilic), low volatility, and excellent effectiveness at small concentrations (Vieira *et al*., 2018). IMI is effective in combating sucking, soil, and chewing insect pests (Lewis *et al*., 2016). Since its introduction, IMI has become the insecticide with the highest sales globally (Casida, 2018). This neuroactive chemical competes with normal nerve impulses by disrupting the nicotinic acetylcholine receptors (nAChR) at postsynaptic junction of insect pest (Mohr *et al*., 2012). IMI dissolves readily in aqueous solution. Stability of IMI is greatly influenced by the pH of water, which has longer half-life and persists in alkaline medium. The half-lives in soil can vary from 174 to 578 days; in water and water-sediment, they are 30 and 129 days, respectively (Petkovic *et al*., 2022). Toxic residues of IMI, have been observed in crops, meals, and surface and even consumable water throughout the world (Table 2). Due to high water solubility, IMI enters the aquatic ecosystem through surface runoff, threatening the non-target aquatic life, including fish (Shan *et al*., 2020).

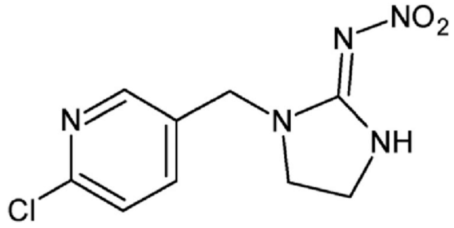

Fig. 1. *Chemical structure formula of Imidacloprid (IMI) (Verebova and Stanicova, 2022)*

Toxic effects of imidacloprid in fishes

Indirect effects

Fish tend to be several orders of magnitude less sensitive to direct short-term exposure of IMI, as seen by the

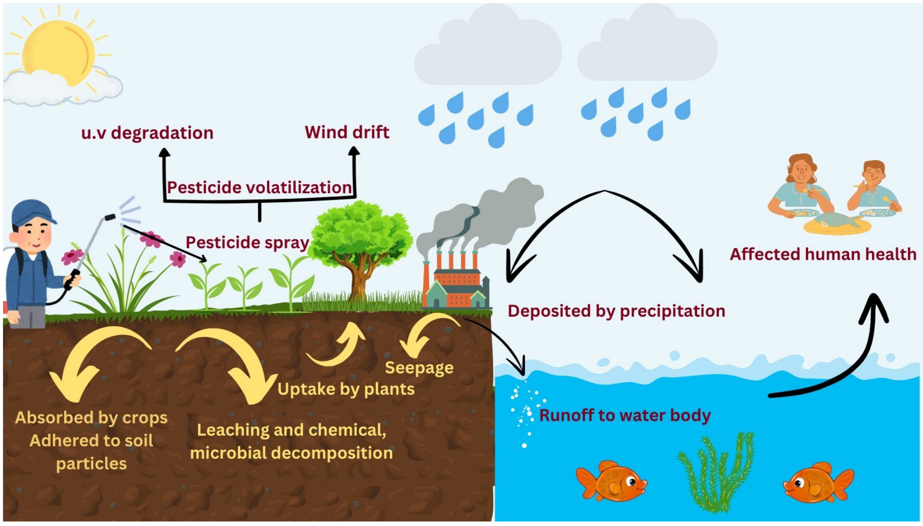

Fig. 2. *Pesticide occurrence in aquatic system (Rajmohan et al., 2020; Pradhan et al., 2022)*

Table 1. Lethal Concentration 50 (LC50) of Imidacloprid (IMI) for a variety of fishes

Name of fish	LC50 of IMI	References
Danio rerio	4.90 – 8.37 mg/L (96h)	Ding *et al*., 2004
Danio rerio	408-1160 mg/L (48h)	Tisler *et al*., 2009
Carassius auratus *Cyprinus carpio* *Ctenopharyngodon idella*	24.8 mg/L (96h) 6.68 mg/L (96h) 13.2 mg/L (96h)	Gradila, 2013
Labeo rohita	0.840 mg/L (96h)	Desai and Parikh, 2014
Labeo rohita	550 mg/L (96h)	Qadir *et al*., 2016
Cyprinus carpio (Eggs)	78 ppm (48h)	Tyor and Harkrishan, 2016
Aequidens facetus	10 ppm (96h)	Iturburu *et al*., 2017
Ctenopharyngodon Idella (Juvenile)	300 mg/L (96h)	Moghaddasi, 2017
Cyprinus carpio (Juvenile)	200 ppm (96h)	Suman *et al*., 2017
Embryo of *Danio rerio* Larvae of *Danio rerio* Juvenile of *Danio rerio* Adult of *Danio rerio*	121.6 mg/L (96h) 128.9 mg/L (96h) 26.39 mg/L (96h) 76.08 mg/L (96h)	Wu *et al*., 2018
Cyprinus carpio (Embryos)	93719 µg/L (24h) 21691 µg/L (48h) 2352.7 µg/L (72h) 1292.6 µg/L (96h)	Islam *et al*., 2019
Danio rerio	0.423 ml/L (24h) 0.352 ml/L (48h) 0.297 ml/L (72h) 0.270 ml/L (96h)	Yadav *et al*., 2020
Oreochromis niloticus (Juveniles)	175.32 ppm (72h)	El-Garawani *et al*., 2021
Clarias gariepinus	2.03 µg/L (96h)	Rahman *et al*., 2022
Crenicichla sancticaroli	2.5 µg/L (24h) 1.25 µg/L (48h)	Queiroz *et al*., 2022
Poecilia reticulate	120.0 mg/L (96h) 122.65 mg/L	Queiroz *et al*., 2022
Cnesterodon decemmaculatus	55.92 mg/L (24h) 47.34 mg/L (48h) 40.93 mg/L (72h)	de Arcaute *et al*., 2023
Clarias gariepinus	64. 88 mg/L (96h)	Erhunmwunse *et al*., 2023
Pethia conchonius	227.33 mg/L (96h)	Dutta *et al*., 2024

aquatic species sensitivity distribution on survival after a few days of exposure (Gibbons *et al*., 2015). It has been suggested that fish may suffer indirect impacts, such as a decrease in the quantity and quality of crustaceans available for food (Chagnon *et al*., 2015). Reduced dissolved oxygen levels in water by promoting algal bloom and macrophytes, which blocked the sunlight, decreased photosynthesis and slowed the breakdown of IMI, thus extending its harmful effects on macro- and micro-crustaceans that indirectly affect fish health (Sumon *et al*., 2018; Gajula *et al*., 2023). An experiment revealed that IMI exposure reduced a large number of zooplankton and other aquatic invertebrates, leading to increased dominance of rotifers and cyanobacteria, which indirectly affected food availability and habitat quality for fish (Yao *et al*., 2021; Cabrera *et al*., 2023). It has also been confirmed that IMI adversely affects the zooplankton population in rice fields, which had an indirect adverse effect on inhabiting fish growth that used to consume the zooplankton species (Hayasaka *et al*., 2012; Suzuki *et al*., 2024).

Direct effects

In general, the direct impacts of pesticides include the instant death of animals or sub-lethal consequences related to development and reproduction, depending on the degree of toxicity (Sanchez-Bayo, 2011). Similarly, Fishes exposed to high concentrations of IMI resulted in instant or delayed mortality. For example, fish *C. gariepinus* exposed to 190mg/l and *L. rohita* exposed to 900mg/l of IMI resulted in heavy mortality of exposed fishes (Qadir *et al*., 2014; Osazee *et al*., 2024). However, at sublethal concentrations, IMI treatment may have resulted in several health impacts including decreased acetylcholinesterase activity, histopathological damage in various tissues, elevated oxidative stress markers, DNA damage in various tissues, altered haemato-biochemical profiles, decreased plasma proteins, albumin, and globulin levels in exposed fishes as described below.

Table 2. Reported concentrations of Imidacloprid (IMI) in surface water worldwide

Countries	Surface water concentration	References
Surface water, Rio grande do Sul, Brazil	126 ng/L	Bortoluzzi *et al*., 2007
California, USA Imperial	3.29 µg/L	Starner and Goh, 2012
Salinas	3.05 µg/L	
Santa maria	1.38 µg/L	
Guadalquivir river basin Spain	2.3-19.2 ng/L	Masia *et al*., 2013
Sydney (Australia)	4.6 µg/L	Sanchez-Bayo and Hyne, 2014
Stream water drains potato field (Eastern Canada)	11.9 µg/L	Morrissey *et al*., 2015
Great smoky Mountains national park (southern Aplanches)	28.5 ng/L	Benton *et al*., 2016
Stream (alum creek)	36.8 ng/L	
(Indian camp creek)	78.0 ng/L	
(Kingfisher creek)	33.6 ng/L	
Southern Ontario (Canada)	230 ng/L	Struger *et al*., 2017
Danube river, Romania tributaries	0.5-8.1 ng/L	Iancu *et al*., 2019
Arges river	3.4- 8.2 ng/L	
Olt river	3.2-3.9 ng/L	
Osaka city Japan	7-25 ng/L	
Seven watersheds, Ontario, Canada	1333 ng/L	Metcalfe *et al*., 2019
Northern Portugal		Sousa *et al*., 2019
Ave river (summer)	480 ng/L	
Sousa river (summer)	208 ng/L	
Sousa river (Autumn)	213 ng/L	
South china (Pearl River)	31.0 ng/L	Zhang *et al*., 2019
Spring	55.7 ng/L	
Summer	180 ng/L	
WWTP effluents	89.1ng/L	
Tengi river, Malaysia	57.7 ng/L	Elfikrie *et al*., 2020
Great lakes basin (Ontario Canada)	1333 ng/L	Wang *et al*., 2021
Taihu lake (china)	41 ng/L	
Wet season	69 ng/L	

Imidacloprid induced behavioural changes in fish

Behaviour is a reaction that demonstrates how external stimuli influence the physiological activities of organisms (Saaristo *et al*., 2018). The behavioural complication studied by many researchers may be linked to IMI impact on the fish Neurological systems through modifying acetylcholinesterase levels that lead to disturbed brain activity (Rahman *et al*., 2022). In *C. carpio* IMI exposure is well known for inducing abnormal behaviour like hyper excitations, erratic swimming, jerky movements, loss of equilibrium, restless, excess mucus secretion, increased mouth gulping for oxygen and operculum motion (Bhardwaj *et al*., 2020a). Other toxic effects include sleepiness, poor swimming, elevated fins, colour darkness, high mortality rates, frequent opercular and fin motions, loss of harmony and lethargic behaviour in *L. rohita* and *O. niloticus* even at the lowest dose of IMI (Desai and Parikh, 2014 and El-Garawani *et al*., 2022). Fish *D. rerio* also exhibited erratic motions and impaired mobility, with freezing behavior in the bottom part of the container on exposure of IMI at a dose of 45 µg/L (Guerra *et al*., 2021). Excessive mucus secretion is another response against IMI toxicity that creates a barrier between toxins and skin, to minimise the risk of contact and its irritating impacts (Patil and David, 2008).

Histopathological alterations

Gills

The gills are an essential component of fish anatomy, carrying out several physiological processes like gas exchange (respiration), waste removal (Excretion) and osmoregulation (Evans *et al*., 2005). Because of their susceptible nature and proximity to the surrounding medium, fish gills are primarily regarded as a mirror of water quality and strong bioindicators of toxicant exposure (Erkmen *et al*., 2017). A number of severe histopathological alterations, including hyperplasia, epithelial lifting, lamellar fusion, oedema, thickening of lamellae epithelium, dilatation of congested capillaries, and central venous sinus in filaments, were observed in gill sections of fish (Nile tilapia) after exposure of IMI (Gunal *et al*., 2020; El-Garawani *et al*., 2022). Similar types of deformities were also reported in fish *O. mossambicus* and *C. carpio* due to exposure to different concentrations of IMI (Patel *et al*., 2016; Ozdemir *et al*., 2018; Harkrishan *et al*., 2020).

Kidney

The kidney serves a critical role in regulating bodily fluid equilibrium and eliminating harmful xenobiotic residues. Numerous investigations revealed that pesticides resulted in various histological changes in the renal tissues of fish (Rohani, 2023). Several structural alterations in the kidney of *C. gariepinus* like swelling of renal tubule and Lymphocytes, balloon necrosis, renal cell depletion, glomerulus distortion, severe necrosis, vacuolation of glandular epithelium, renal tubular and sinusoidal enlargement, tissue degeneration, renal tubular necrosis, exudates in tubules, necrosis of single cell, renal epithelium desquamation, and degenerated renal tubules etc. were observed after exposure to IMI (Kurikose *et al*., 2022). Research conducted by Kochetkov *et al*. (2023) reported some IMI induces changes in the kidney of *D. rerio,* like vacuolization of the cuboidal epithelium of the proximal tubules, increased Bowman's space and renal corpuscle area, thickened proximal tubule epithelium, cell debris within the inflammatory areas, mononuclear leukocytes, focal inflammatory foci contained epithelioid macrophage cells containing large nuclei. Similarly, IMI also causes various alterations in renal tissue of exposed fish, including necrosis, vacuolation, pyknosis of the nucleus and swelling in the tubular lumen, infiltration of W.B.C and growing hematopoietic tissues, tubular epithelial condensation, fusion and fuzziness of cell margins, karyorrhexis and karyolysis (Qadir and Iqbal, 2016).

Liver

The liver of fish functions as the primary organ for multiple metabolic reactions along with alterations in liver histology, and it is mainly essential for detoxification, enzyme synthesis, and biochemical processes, which serves as a crucial biomarker in ecotoxicity studies of fish (Erkmen *et al*., 2017; Sharma *et al*., 2022). Gunal *et al*. (2020) reported IMI-induced deformities in the liver of *O. niloticus,* including increased lipid accumulation, passive hyperemia, and mononuclear cell infiltration, causing inflammation and vacuolization. However, necrosis of hepatic tissues and hydropic deterioration were observed in *C. carpio* when exposed to a high dose of IMI at 140 mgL^{-1} (Ozdemir *et al*., 2018). Several liver alterations such as leukocyte infiltration, vacuolation of cytoplasm, hemorrhage, liver cell necrosis and central hepatic vein wall dilatation and congestion were all observed in O. niloticus IMI exposure (Ramirez-Coronel *et al*., 2023; Al-Awadhi *et al*., 2024). Some hepatic alterations were also revealed in *D. rerio*, including foci of necrosis near the bile ducts, cells with cariorexis, leukocyte infiltration, PAS-positive granules and enlarged nuclei of hepatocytes. These changes indicate an increased detoxification response to the presence of IMI (Kochetkov *et al*., 2023). In addition, pyknotic nuclei, hepatocyte loss, hydropic degeneration by accumulation of water and electrolytes inside the cell, nucleus dislocation and blood sinusoidal dilatation were noted in various fishes exposed to IMI at different doses (Qadir and Iqbal, 2016 and Ansoar-Rodriguez *et al*., 2016).

Intestine

Like other toxicants, IMI also disrupts the digestive processes of fish by causing distinct alterations in the muscularis, mucosa, submucosa, and villi membranes of intestine. Fusion and hyperplasia of enterocytes, disruption in villus morphology and lamina degeneration, bifurcation and inflammatory cell infiltrate, vacuolization and necrotic cells in muscularis externa of intestine observed in IMI treated zebrafish (Akbulut and Ertug, 2019). Alterations like McKnight cells (apoptotic) in some areas of epithelium, excess presence of Rodlet cells and intraepithelial leukocytes clusters in the mucosa, lowered height of adsorbing epithelium and increased thickness of the lamina propria were also observed in renal tissue of *D. rerio* (Kochetkov *et al*., 2023). Miao *et al*. (2022) investigated specific changes in the common carp intestine, observing the lack of microvilli and columnar cell disorganization, contributing to reduced submucosal thickness. Uplifted mucosa, inflammation, distorted muscles and mucosa, edema, hemorrhage, atrophy, and necrotic bodies were observed in intestinal tissue of *C. carpio* after IMI treatment at low to high doses, which indicated several abnormalities in gut (Kamboj *et al*., 2024). All these alterations disrupt the function of the intestinal barrier and gut permeability, reduce villus heights and increase mucus secretion, which could further affect the status of nutrient absorption (Luo *et al*., 2021).

Gonads

Exposure to pesticides, including IMI results in various deformities in reproductive tissues that adversely affect the reproductive performances of exposed fish. For example, histological changes like interstitial fibrosis, hypertrophy in Leydig and spermatogenic cells, destruction of seminiferous tubules in the testicular tissue of fish (Kapoor *et al*., 2011; Akbulut, 2021). Moreover, a variety of abnormalities like oocyte structural degradation, autolysis, increase in atretic oocytes, curling and thickening of zona radiata, vacuolization in cortical alveoli and perifollicular layers opening seemed common in ovarian tissue of IMI exposed fish (Akbulut, 2021).

Similarly, IMI exposure in *M. anguillicaudatus* also resulted in disorganized lobules with expanded gaps, leading to reduced ratios of spermatozoa and spermatids (Xia *et al*., 2016).

Nervous system

Nervous tissue is vital for maintaining control and coordination; therefore, its integrity and functioning are very important. IMI is a neurotoxic chemical and its exposure induces neuronal degeneration, vacuolation, necrosis, enlarged neurocytes, pyknotic nuclei, spongiosis, mononuclear infiltration, and fragmented nuclei in cerebrum and optic lobe of *C. carpio* (Gernhofer *et al*., 2001; Harkrishan *et al*., 2020). Similarly, Zebrafish (*D. rerio*) exposed to imidacloprid experienced disturb social act like mutual attentiveness and attraction due to histological alteration in optic tectum of brain, oxidative stress, inflammation and apoptosis of nerve cells (Chung *et al*., 2023).

Early developmental aspects

Exposure to toxicants during early development disturbs the process of normal development and induces disorders as well as impairment that reduce the survival chance of exposed fish. Initial developmental stages of fish show a significant vulnerability to widespread toxicants including IMI (Tyor and Harkrishan, 2016). IMI creates great risks for non-target aquatic life, including fish, by affecting embryological developments due to its genotoxicity (Dutta *et al*., 2024). A number of defects, including malformed tail with unhatched embryo, yolk-sac swelling, impaired body structures and head forms, have been reported in the embryos of *C. carpio* treated with varied concentrations of IMI (Islam *et al*., 2019). In addition, undeveloped bodies like coma and oval-shaped, vertebral axis curving, a single-eyed hatchling, distorted head, a flexure tail, and distorted yolk sac were also common in larvae of *C. carpio* exposed to IMI (Tyor and Harkrishan, 2016). IMI exposure also induces a reduced growth rate with the high mortality rate in fish during the early stage of development (Bhardwaj *et al*., 2022). Teratogenic effects of IMI on fish larvae include aberrant spinal curvature, defective jaws and skull cavity, profuse bleeding, lordosis or scoliosis, inflammation of the yolk, abnormal bones and tail, yolk sac retention, poorly developed eyeball and barbels, lowered hatching rate along with decreased cardiac output (Erhunmwunse *et al*., 2023). IMI also induces pre-mature hatching and reduces the hatching period in the exposed eggs up to four hours (Islam *et al*., 2019; Bhardwaj *et al*., 2022).

Haematological aspects

The hematologic index is a useful tool for evaluating different blood parameters and determining the sublethal effects of contaminants on the health of aquatic species, including fish (Witeska *et al*., 2023). Similar to other pesticides, low dosages of IMI that are insufficient to kill fish in spite induces haematological alterations frequently (Table 3). Changed and reduced leucocyte count is a general response that impairs the immunological defense of stressed fish (Rahman *et al*., 2022a; Razaa *et al*., 2023). In addition, intense anaemic conditions with declined haematological parameters like haemoglobin, erythrocyte, PCV, MCV, and platelet levels are another response induced by sublethal dose of IMI, which reduces their formation in stressed fish (Saaristo *et al*., 2018; Rahman *et al*., 2023; Gajula *et al*., 2025). Declined hematological parameters, Anemic changes and disruptions in osmoregulatory systems could result from adverse effects caused by pesticides on the blood-forming organs, resulting in erythrocytic damage and imbalanced blood cell formation rate (Bhardwaj *et al*., 2020; Shahzadi *et al*., 2024). In addition, IMI toxicity also induces a notable decrease in the plasma protein content of exposed fish (Americo-Pinheiro *et al*., 2019).

Biochemical aspects

Exposure to IMI also causes chemical stress, leading to altered biochemical processes and even causing qualitative and quantitative impairment in proteins, lipids, nucleic acid, and even cell (Vieira et al., 2018; Guerra *et al*., 2021). Toxicity of IMI induces significant reduction in exposed fish protein content in the muscles and liver. Enhanced catabolic reactions and over utilization of amino acids under stress conditions are the main causes of the reduction in protein content (Vishal *et al*., 2012). Declined protein content indicates the increased rate of proteolytic action or protein breakdown in order to fulfil the energy demand (Vijayan *et al*., 2018). However, over time, exposure to IMI increases the chemical stress, resulting in declined composition of amino acids such as glycine, serine, and arginine in exposed fish (Qadir *et al*., 2017). IMI treatment also induces a remarkably rise in some serum protein levels, like creatinine, and others, such as globulin and albumin, in fishes like groups of *C. idella* and *C. auratus* (Ilahi *et al*., 2018). Elevated glutathione S-transferase (GST) in brain cells of fish *D. rerio* along with liver and muscle tissues of *O. niloticus* after exposure to IMI indicate protein damage (Guerra *et al*., 2021; Temiz and Dayangac, 2024). In addition to alterations in protein content, IMI exposure also causes a decline in the glycogen level in liver and muscle tissues that might result in direct overconsumption of glycogen content for energy generation under stressed conditions (Ismayil and Joseph, 2020). Increased malondialdehyde (MDA) content levels and ROS generation is another biochemical alteration induced by IMI in treated fish (Ge *et al*., 2015). Catalase (CAT) and Superoxide dismutase (SOD) are key enzymes acting as primary oxidative defense systems, were markedly elevated in the gut of *D. rerio* along with the residual product of fatty acid peroxidation, *i.e.* malondialdehyde (MDA) after IMI exposure which indicating oxidative damage (Hong *et al*.,

Table 3. Haemato-biochemical alterations due to Imidacloprid (IMI) toxicity in different fish species

Species	Dose	Effects	References
Cyprinus carpio	40ppm	High leucocyte count, drop in Hb level, increase clotting time	Suman *et al*., 2017
Ctenopharyngodon idella	2ppm	Increase neutrophil, Decreased platelet count, drop Hb level	Ilahi *et al*., 2018
Carassius auratus	2ppm	Elevate T.L.C level, lymphocyte count, Declined platelet count and Hb level	Ilahi *et al*., 2018
Oriochromis niloticus	0.01 µg/l 0.05 µg/l 14.50-28.1 mg L^{-1}	Declined RBCs counts, Hb concentration, and HCT levels, increased WBCs, Elevated MCV and MCH values	Americo-Pinheiro *et al*., 2019; Naiel *et al*., 2020; Abdel-Tawwab *et al*., 2021
Clarias batrachus	3ppm	Decline in haemoglobin level, Decreased R.B.C, PCV, MCV, MCH Rise in W.B.C count	Tokriya and Billore, 2024
L. rohita	66.6 mg/l	Declined haemoglobin, RBC and HCT level, increased Leucocyte count	Gajula *et al*., 2025

2020; Luo *et al*., 2021). However, Numerous studies have reported that prolonged exposure to IMI leads to a decrease in the levels of CAT and SOD in the hepatic tissue of *O.* niloticus, indicating that the decline in their activities may be attributed to toxicity-induced oxidative stress occurring at a later stage resulting in liver damage (Attia *et al*., 2021; Huang *et al*., 2023).

Hong *et al*. (2018) reported that IMI exposure resulted in decreased hepatic ALP activity which might disturb the membrane characteristics and reduced CRP and C3 (complement proteins) levels resulting in impaired detoxifying capacity of fish liver. It has also been reported that exposure of IMI induces significant oxidative damage or lipid peroxidation in both gill and liver tissue of fish *O. niloticus,* to which young fish are more prone (Vieira *et al*., 2018 and Gunal *et al*., 2020). Elevated hepatic enzymes like ALT, ALP, AST, and LDH, along with reduced SOD and CAT significantly resulted in the hepatic injury of *C. carpio* (Ramirez-Coronel *et al*., 2023). Similarly, IMI exposure to *C. gariepinus* and *O. niloticus* also showed a significant increase in ALT and AST enzymes in blood that reflects the hepatic stress due to ROS generation (Abdel Rahman *et al*., 2022; Subaramaniyam *et al*., 2023; Al-Awadhi et al., 2024). Immunological response to exposure to IMI involves a remarkable decrease of serum IgM and lysozyme action, resulting in declined innate immunity of stressed fish (Hong *et al*., 2018; Ramirez-Coronel *et al*., 2023).

Genotoxic aspects

Genotoxicity of a substance can be measured in terms of its capacity to induce destructive alterations in the genetic material like DNA or mRNA, leading to genetic mutations and damage to the genome. Substances with genotoxic properties damage the DNA and also cause disruptions in cellular structures and activities (Muazzam *et al*., 2019). Genotoxic impacts of IMI, have been tested on several fishes like *M. Anguillicaudatus*, *P. Lineatus*, *A. facetus* (Xia *et al*., 2016; Iturburu *et al*., 2017; Viera *et al*., 2018). Alvim and Martinez (2019) reported nuclear membrane destruction and DNA damage in the liver, gills and RBC's of *P. lineatus* after exposure of IMI, resulting in higher ENA (Erythrocytic nuclear abnormalities) formation. Almeida *et al*. (2021) also reported similar observations like ENA, SN (segmented nuclei) and KN (kidney-shaped nuclei) in *A. altiparanae* when treated with IMI. IMI exposure causes Nuclear aberrations such as chromosome fragmentation in dividing cells and contribute to the accumulation of micronuclei (MN) and ENA formation, indicating as a crucial biomarker of genotoxicity (Ispir & Ozcan, 2021; El-Garawani *et al*., 2022).

Similarly, IMI exposed fish (*O. niloticus*) also exhibit increased MN and ENA frequency, might be a result of mitotic spindle malfunction or broken nucleic acid strands of the hematopoietic tissues (El-garawani *et al*., 2021). Research reported that DNA damage in the liver of *O. niloticus* after IMI exposure is caused by unfolding and disruption of genetic material, resulting in genotoxicity (Ramirez-Coronel *et al*., 2023). Single strand breaks, alkali-labile sites, increased length or moments of the olive tail and delayed-repair sites in exposed fishes also indicate potential genotoxicity of IMI (Ansoar-Rodriguez *et al*., 2015; Muazzam *et al*., 2019). Decreased percentage of head DNA, increased Tail DNA, Tail length and olive moments indicated the DNA damage in RBC's of *C. carpio* followed different IMI exposures (Kamboj *et al*., 2024).

POSSIBLE CONTROL MEASURES

Integrated Pest Management (IPM)

Insects, weeds, and viruses adversely impact global agriculture, resulting in a remarkable loss in agricultural output worldwide. When evaluated against food security, yield declines from pests may equal the food required to feed almost a billion people. IPM is a multidisciplinary approach that keeps insect numbers below a threshold that cannot negatively impact the economy. Integrated pest control is a way of pest management through chemical and biological control operations (Stern et al., 1959). The idea of "Integrated Pest Management" was initially applied to agriculture in the 1970's to reduce the harmful consequences of pesticides on non-target organisms. IPM acts as a shield to offer a low-risk, broad, and efficient method of securing both people and assets against pests (Anonymous, 2004). Several pest-control techniques like, cultural, biological, genetic, physical, legal and mechanical, are incorporated into a single program using this methodical approach to reduce pesticide usage. Some of these methods are given below.

Physical and mechanical control

Physical control is modifying the surroundings to prevent insect pests from thriving or growing. The two types of physical methods are passive (Hermetic storage, packing, air doors, screening) and another one is active (inert dust, sieving, temperature adjustment (aeration treatments, heat, grain cooling, etc.) and electromagnetic methods, *i.e.* microwaves, radio frequencies, and ionizing rays (Angon *et al*., 2023). Pest control techniques like temperature, moisture and humidity manipulation, irradiation and flooding regulate insect life cycles, control microbes, disinfect soil, and influence insect behaviour and physiology. Additionally, light, sound waves, flaming, and traditional noise methods have been historically used in agriculture for pest management (Adhikari, 2022). The most commonly used manual pest-eradication strategies are mass shooting, mass trapping and mating disruption (Yamanaka, 2007). Several other tools like fences, trenches for crawling insects, sticky tape on trees, pheromone traps, funnel traps, sucking traps, wing traps, water pan traps, methyl eugenol traps, pitfall traps, etc., can also be utilized for effective pest control (Dara, 2019).

Biological control

Recent IPM studies have focused on biological control through conservation measures and innovative biopesticides that enhance the role of natural enemies and pest control using botanical and microbial agents (Zhou *et al*., 2024). Biological control is the application of natural enemies to control undesirable and harmful plants (weeds), insects and animals (Baker *et al*., 2020). These natural enemies can be termed biopesticides such as bacteria, fungi, viruses, parasitoids, pathogens, predators, herbivores that feed on weeds, entomopathogenic nematodes and hyperparasites of plant pathogens that can effectively control pests without harming other creatures, replacing conventional pesticides (Heimpel and Mills, 2017; Sood P, 2024). The effectiveness of natural enemies depends on their reproductive capacity, host specificity, environmental adaptability and ability to locate pests (Bielza et al., 2020) efficiently. Parasitoid creatures are highly specific kill their prey and develop entirely on a single host (Salim *et al*., 2016) Recent studies indicated that parasitoids from different families like Ichneumonidae, Bethilidae, Pteromalidae and Braconidae have acted against pests of stored food (Harush *et al*., 2021). Entomopathogenic nematodes (soil worms) like *S. carpocapsae, H. heliothidis, H. bacteriophora, H. zealandica* are capable to control soil born pests like billbugs, armyworms, chinch bugs, fungus and scarab grubs (Dara, 2017; Angon *et al*., 2023). Further, introduction and encouragement of predatory vertebrates like fish, amphibians, reptiles and birds also effectively control harmful insect pests (Heimpel and Mills, 2017; Tanda, 2024). Herbivorous fish like rohu, tilapia, grass carp, punti, niloticas, etc. can be used to eliminate undesirable plants (aquatic weeds) like duckweeds from an aquatic ecosystem (Mandal and Bera, 2024). Certain pathogenic arthropod species effectively restrict the growth of exotic weeds up to 83% (Hayes *et al*., 2013; Fowler *et al*., 2024).

Selection of pest-resistance crops

Pest-resistant crops are genetically modified or selectively bred to reduce damage caused by pests, including fungi and viruses. Such crops are unsuitable for insects regarding egg laying or subsequent development. Along with desirable economic traits, such plants can withstand the infection or reduce pests to a level that causes insignificant damage to plants during growth (Dara, 2019). Resistant crop varieties play an essential role in IPM by lowering pesticide reliance, reducing yield losses, and increasing the durability of crops through strategies such as antixenosis, antibiosis, and tolerance (Mani *et al*., 2022). Antixenosis prevents pests from eating, ovipositing, or hiding themselves, while antibiosis inhibits their development, reproduction, or longevity by some poisonous or physical obstacles and tolerance enables plants to endure or recover from damages via increasing stress tolerant ability (Zhou *et al*., 2024). Bt cotton is a good example of a resistant crop, significantly reducing pesticide dependence while effectively controlling lepidopteran pests like the cotton bollworm (Razzaq *et al*., 2023).

Antioxidants against Imidacloprid (IMI) toxicity

Various compounds such as n-acetylcysteine, vitamins (A, C, E and D), omega-3 fatty acids and coenzyme Q10 are well known for their significant protective effects against toxicity induced by pesticide exposure. These substances possess anti-inflammatory properties, enhance detoxification process, and reduce oxidative stress and cellular damage caused by pesticides (Sajad *et al*., 2024). Antioxidant compounds present in various plants' extract also reduce the detrimental effects of pesticides on non-target organisms. Compounds such as phenolic, curcumin, resveratrol, and flavonoids have been known for scavenging free radicals (ROS). Quercetin is a natural plant-derived flavonoid compound found in many fruits and vegetables. Different investigations have confirmed that quercetin is a potential antioxidant against imidacloprid toxicity (Moniem *et al*., 2019; Miao *et al*., 2021). The aqueous extract of *Moringa oleifera* leaves reduced IMI-induced oxidative stress by lowering the synthesis of ROS in zebrafish (Yadav *et al*., 2020). Meanwhile, Naiel *et al*. (2020a) reported that vitamin C and chitosan nanoparticle-enriched diets were found to have antioxidant qualities that prevent *O. niloticus* against IMI toxicity. *Hyphaene thebaica* fruit (HTF)-enhanced diet improved blood-antioxidant levels in *C. gariepinus*, as its flavonoids and polyphenols scavenged free radicals, preventing lipid peroxidation and ROS stress induced by IMI (Rahman *et al*., 2023). Similarly, Caffeic acid phenethyl ester (CAPE) is another phenolic compound that occurs naturally in plants and can be used as a therapeutic agent against pesticide-induced liver toxicity (Shao *et al*., 2020). Antioxidant and anti-inflammatory properties of turmeric and ginger have been found to diminish pesticide-induced liver and kidney injuries. Supplementation of *Allium sativum, Citrullus colocynthis, Moringa oleifera, Thymus vulgaris*, Menthol oil, *Panax ginseng, Origanum majorana* and *Origanum vulgare* also shows significant ameliorative effects against toxicity of various pesticides (Mehrandish *et al*., 2019; Ahmed *et al*., 2022; Mahmoud *et al*., 2022).

Future prospective

Research on biomagnification in aquatic food chains is needed to be assessed thoroughly. Further, advanced molecular and genetic studies can help uncover imidacloprid toxicity, particularly its impact on fish reproduction, growth, and immune system at the cellular level. Future research should also explore how global warming influences imidacloprid degradation, persistence, and susceptibility towards fishes. Imposing regulatory laws towards the limited use of imidacloprid near aquatic ecosystems can help to mitigate risks for aquatic life. Dietary intake of antioxidant compounds present in various plants extract should be promoted to mitigate the detrimental effects of pesticides on non-target organisms. Future policies may focus on educating farmers about the environmental risks of imidacloprid and promoting sustainable pest control methods, such as integrated pest management (IPM) that can reduce pesticide runoff into aquatic environments.

Conclusion

Imidacloprid is an aquatic contaminant that frequently surpasses many current standards for water quality limits. It can negatively impact the development, survival, activities and behaviour of several untargeted fish populations at various life stages. This review concludes that IMI treatment triggers alterations in developmental physiology, hematology, impaired body structures, behavioral impairments, biochemical parameters or enzymatic pathways with oxidative stress conditions and other genotoxic consequences in different treated fishes. Thus, there is a need to reduce IMI contamination by using organic farming to implement natural solutions and IPM strategies for preventing pest incursion. Therefore, the vast utilization of IMI should abstain near aquatic ecosystems and a need for stringent regulatory considerations for future applications. Moreover, intense research should be carried out to find a potential way to control IMI contamination in aquatic ecosystems. In addition, dietary supplementation of various antioxidants should be recommended as a therapeutic agent against the toxicity of pesticides, including imidacloprid.

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