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Toxicological Impact of Malachite Green on Freshwater Fish: A Comprehensive Review

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Authors' contributions

This work was carried out in collaboration among all authors. Author RB and MAH designed the study and wrote the first draft of the manuscript. Author SSR managed the literature searches and wrote the final draft. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Triarylmethane dyes, such as malachite green, are widely used in the culinary, pharmaceutical, textile, and other sectors for a variety of purposes, including aquaculture parasiticides. It controls fungal attacks, protozoan infections, and some other diseases caused by helminthes on a wide range of fish and aquatic organisms. Nonetheless, the dye's purportedly harmful effects have raised a lot more questions about its usage. The exposure time, temperature and the concentration all raise the dye's toxicity. It has been linked to chromosomal breaks, mutagenesis,

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cancer,teratogeneity, and pulmonary toxicity. Multi-organ tissue damage is one of malachite green's histopathological consequences. Fish exposed to malachite green have notable changes in their blood's biochemical characteristics. Malachite green and its reduced form, leucomalachite green, have been found in a variety of tissues, including eggs and fries, as well as serum, liver, kidney, and muscles. Despite a wealth of information about malachite green's toxicity, several developing countries still utilize it as a parasiticide in aquaculture and other industries. Hence, there is a significant likelihood of malachite green disposition in the food chain. To counteract its toxicological effect, it is crucial to identify a substitute because of its high toxicity.

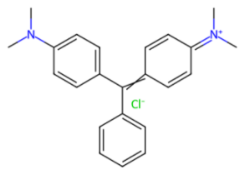
Keywords: Toxicity; teratogenecity; malachite green; cat fish; parasiticide; dye compound.

1. INTRODUCTION

The wide spread use of pesticides by human beings to control pest, diseases in various fields like agriculture, forestry, aquaculture and other public gardens has changed the ecological balance of the environment in various aspects. Many of these pesticides have long been known to be toxic to aquatic ecosystem. A new era of pest control was ushered in with the introduction of synthetic pesticides of a higher generation; however the high biochemical toxicity of these compounds to living things has led to environmental issues. Since very few pesticides are considered to be unique to a particular insect or illness, many other forms of life are commonly harmed by their actions. Fish are especially sensitive to many different pesticide chemicals, and if these pesticides are used excessively in regulated aquaculture methods, they may also leak or be purposefully discharged into rivers and lakes, creating harmful circumstances [1,2,3]. The wide-spread use of these chemicals is greatly responsible for considerable increase of pollution to cultivable waters and has posed a great threat to the whole of the aquatic community including fish. Fish in particular are typically affected by changes in the natural aquatic environment's chemical composition on their physiological and behavioral systems [4,5,6]. This results in toxicological effect on behavioral, physiological and biochemical processes by entering into some organs affecting mainly the liver declining in protein content. In many developing nations, fish are widely raised in inland waterways because they are thought to be the most nutritious form of animal protein. Omega-3 fatty acids, calcium, phosphate, vitamin D, and protein are among the nutrients found in fish [7]. Due to pesticides' entry and disposition in a variety of fish tissues, it is imperative to investigate the harmful impact of these substances on alterations in haematological, histopathological, biochemical, and cellular qualities. Among various pesticides, malachite green, a triarylmethane dye (N-methylated diaminotriphenylmethane dye) is primarily used in aquaculture as a parasiticide or fungicide is well known (Table 1). Foster and Wood Bury (1936) employed malachite green as a dip treatment to suppress fungus, which was the first documented application of malachite green in fish culture more than 80 years ago [8]. Moreover, malachite green is employed as a biological stain, a medicinal disinfectant and antihelmintic [9,10] (Nelson, 1974) a pigment in the ceramics industry, an additive in the paper industry, and a direct dyer of silk, wool, jute, and leather [11]. Studies reveal that malachite green is widely used as a food colouring additive in milk products and other food items, despite being prohibited in the majority of countries, including the US [12,13]. Malachite green is frequently used to cure adult fish, while its primary purpose in fish culture has been to prevent oomycetes fungi from growing too much during the incubation of fish eggs [14,15,16]. Malachite green is used as an aqueous solution, usually sporadically, in tanks, troughs, ponds, or raceways to treat these and other conditions. As a result, malachite green is quickly released into the aquatic environment [17].

A wide variety of malachite green concentrations are used to treat fungal and parasite illnesses; dosages ranging from 100 ppm for a brief dip to 0.1 ppm for an ongoing therapy in ponds [10,18]. Malachite green's purportedly harmful effects have raised a lot of questions about its usage, nevertheless. As exposure to this insecticide rises in temperature and concentration over time, it becomes increasingly dangerous. It has been connected to chromosomal breakage, mutagenesis, carcinogenesis, and respiratory toxicity. Multi-organ tissue damage is one of malachite green's histopathological consequences. Blood biochemical markers such as total leucocyte count (TLC), total erythrocyte count (TEC), and haemoglobin concentration significantly change in fish exposed to malachite

Table 1. Malachite green: Summary

Summary of Malachite Green			
Sl. No.	**Particulars**	**Inference**	**Reference**
1.	Structure		CSID:1820 (ChemSpider,2024) [20]
2.	Molecular Formula	$C_{23}H_{25}N_2.Cl$ $C_{23}H_{25}ClN_2$	PubChem, NCBI(2024) [21]
3.	Synonyms	569-64-2 Basic Green 4 Malachite Green Chloride China Green	PubChem, NCBI(2024) [21]
4.	Molecular Weight	364.9 g/mol	PubChem, NCBI(2024) [21]

green. There have been reports of leucomalachite green and malachite green residues in eggs and fries, as well as in blood, liver, kidney, muscles, and other tissues [19]. Recent studies shows malachite green exposed fish shows genotoxicity like micronuclei and other abnormalities like lobed nuclei, notched nuclei, distorted nuclei. Malachite green still serves as a parasiticide in aquaculture and other sectors of the economy, despite a wealth of information about its hazardous effects. Until those changes, malachite green should be used extremely carefully, at the right concentration, and at moderate temperatures. In this communication, the toxicological effect of malachite green is described in a fresh water fishes inhabiting in various natural habitats like swamps, ponds and brackish water systems of North-east India.

2. APPLICATIONS OF MALACHITE GREEN BEYOND AQUACULTURE

In the aquaculture sector around the world, malachite green is a widely utilized biocide. In significant protozoan and fungal infections, it is quite effective [22,23,24]. It has been used to manage skin and gill flukes and essentially acts as an ectoparasiticide. Malachite green has been widely used by the aquaculture industries as a topical treatment using bath or flush procedures, but these has been done without considering the possibility that therapeutants administered topically could also be absorbed systematically and have major interior effects. However, it is also utilised as a food colouring, food additive, anthelmintic, medical disinfectant, and dye in the acrylic, silk, wool, jute, leather, cotton, and paper industries [25]. But because of the concerns it presents to fish consumers who consume treated fish—such as genotoxic effects and impacts on the immune system and reproductive system—malachite green has emerged as a highly contentious dye ingredient [26,27,28,29]. Despite being outlawed in a number of nations and lacking FDA approval, this dye is nevertheless widely used because of its affordability, accessibility, and effectiveness. The wide spectrum of biological impacts it exerts on people and other animals is the subject of extensive research. Numerous authors have conducted outstanding research to determine how this chemical affects the biochemical, haematological, behavioural, histopathological, and genotoxic features of aquatic creatures, such as different species of fish. Some of these studies are noteworthy [30,24].

3. TOXICOLOGICAL EFFECTS OF MALACHITE GREEN ON FISHES

LC_{50} values for numerous commercial dyes have been estimated by a number of workers on fish at various time intervals. Some have suggested that it is difficult to evaluate the toxicity of different toxicants since different fish species are impacted by different factors such temperature, pH, hardness, and dissolved oxygen in test water [31,32,33,34]. Bills *et al.* (1977) conducted a comprehensive study to determine the LC_{50} values of malachite green on adults and fingerlings of various fish species. They also observed the impact of temperature, pH, and exposure duration on the toxicity of the dye. Their research shows that when temperatures rise, malachite green's toxicity increases because it accelerates the uptake and accumulation of the dye in tissues [35]. Alderman and Polglase (1984) have also made similar observations [36]. Additionally, Srivastava *et al.* (1995) noted variations in the LC_{50} values of malachite green in *Heteropneustes fossilis*, a freshwater catfish, at various exposure times and began to suggest that toxicity rises with exposure time [37]. Other than death, biological consequences have been the subject of numerous researches. Rainbow trout were treated with 1.6 ppm malachite green for 40 minutes once every seven days for seven weeks, according to Gerundo *et al.* (1991). Most livers showed a reasonably consistent pattern of growing pathological alterations following the third exposure, including diffuse degenerative abnormalities, cytoplasmic vacuolation, sinusoidal congestion, and localized coagulative necrosis [15]. Because of this dye's respiratory poisoning effect, mitochondrial damage was visible at the ultra structural level [37]. Lesions and necrosis were typically seen in the gills, with the latter being more noticeable after longer exposure times. Wright (1976) assessed the largemouth bass (*Micropterus salmonides*) fry and eggs exposed to malachite green mortality rate. The mortality rate of eggs and fries increased by more than 20 times with a two-fold increase in malachite green concentration. He came to the conclusion that malachite green is highly hazardous and shouldn't be utilised for any reason involving large mouth bass fry or eggs as a result of this finding [38]. This dye is extremely hazardous to freshwater fish, according to numerous studies, both in short-term and long-term exposures [39,40,41,42,43,25]. There have also been reports of chromosomal fractures, carcinogenesis, mutagenesis, teratogenecity, and decreased fertility in rainbow trout after malachite green treatment [44,45,46,41,39,34,9]. Research on the teratogenic effects of malachite green on fish has been conducted in significant quantities. Long-term intoxication with malachite green has been linked to significant developmental defects in eggs, primarily chromosomal breakage, in rainbow trouts (*Oncorhynchus mykiss*) [47,39]. Worle (1995) has also reported on chromosomal abnormalities in the eggs of freshwater fish treated with malachite green [48]. Evidence of abnormalities in the spine, head, fin, and tail of rainbow trout fry generated from eggs has been seen, along with a significant decrease in embryo survival after 38 hours of fertilization following extended exposure to elevated levels of malachite green [41].

4. BIOCHEMICAL AND HAEMATOLOGICAL EFFECTS OF MALACHITE GREEN ON FISHES

Malachite green has been shown to significantly change biochemical parameters in the blood of *Heteropneustes fossilis* at acute, sub-acute, and sub-lethal doses, despite Bills and Hunn's (1976) denial that the pigment had any negative effects on the blood chemistry of Coho salmon fish [49,50,36]. Along with raising the blood cholesterol level in catfish and lowering the calcium and phosphorus levels in plasma in tilapia, it also results in the depletion of serum protein and calcium levels [36,51]. After being exposed to malachite green, catfish have been shown to exhibit disruptions in their osmoregulation and metabolism of carbohydrates. Malachite green increases sensitivity to hypoxia, limits some fish's ability to synthesise proteins, and stimulates the breakdown of glycogen in the liver and muscles while simultaneously raising blood sugar and chloride levels [50,52]. Anaemic reactions and drops in haemoglobin levels have been observed in rainbow trout and *Clarius gariepinus*, two more fish species that have been shown to be impacted by malachite green [53,54]. Additionally, after being exposed to malachite green, *Heteropneustes fossilis* showed delayed blood coagulation, an increase in WBC count. It also decreases RBC count along with hemoglobin and HTC% [55]. Following exposure to malachite green, there have also been seen reductions in monocyte count, haemoglobin value, and mean corpuscular volume as well as an increase in mean corpuscular haemoglobin concentration [52]. But rainbow trout exposed to malachite green have been found to have higher haemoglobin and packed cell volume values [25]. Within three days, fish treated with malachite green showed a rise in haemoglobin levels and erythrocyte counts; nevertheless, erythrocytosis and leucopenia were discovered after seven and twenty-one days, respectively [56].

5. HISTOPATHOLOGICAL EFFECTS OF MALACHITE GREEN ON FISHES

The effects of malachite green on the liver, gills, kidney, gut, gonads, and pituitary gonadotropic cells have been demonstrated by histopathology. Additionally to damaging mitochondria and causing nuclear changes, it induces sinusoidal congestion and localised necrosis in the liver [15]. After being treated with malachite green, *Heteropneustes fossilis* hepatocytes showed signs of hypertrophy and vacuolization, which were followed by necrosis and cirrhosis [57]. In addition to causing necrosis of lamellar cells and gill epithelium, exposure to this dye also severely damages gills, causing leucocyte infiltration in rainbow trout and *Heteropneustes fossilis*. The dye induces hyperproliferation of the epithelial cells in the proximal convoluted tubules, shrinkage of the glomeruli, nercotic changes (karyorrhexis, karyolysis, pyknosis, and the desquamation and degeneration of the epithelial cell lining), cytolysis, and an increase in the number of goblet cells, rupture of the tip of the intestinal villi, disruption of the mucosal folds, necrosis, and disorganisation of the muscularis and serosa [58]. There have also been reports of degenerative alterations in the gonads and inhibition of gonadotropic cell activity in the pituitary gland after both acute and chronic exposure to sub-acute and sub-lethal concentrations of the dye in the catfish [59].

6. EFFECTS OF MALACHITE GREEN ON EPIDERMIS OF FISHES

In the epidermis of *Mystus vittatus* and *Anabas testudineus* exposed to varying lengths of malachite green treatment, there is a noticeable rise in the number of mucous cells, which is connected with the increased production of mucus [60]. Increased mucous secretion by the mucous cells, which create a thick, slimy film over the surface of the epidermis at different intervals after malachite green treatment, is important in *Mystus vittatus* and *Anabas testudineus* because it serves to postpone the toxicity of the irritant present in the environment [61]. Appreance of microridges due to the folded superficial layer epithelial cells in *Mystus vittatus* in early duration of malachite green treatment is very interesting [60]. Fish with differing roles for the microridges on their epidermal surfaces have been identified. These include keeping mucous secretion on the cell surface to offer reserve surface area for stretching, to help disperse mucus away from goblet cells to aid in the production of laminar flow, and to enhance surface area for excretion and absorption through skin [62]. Exfoliation of the superficial layer epithelial cells in the epidermis of *Anabas testudineus* and *Mystus vittatus* after varying stages of malachite green treatment is also noteworthy. It appears that these cells are degenerating in different stages in response to the malachite green treatment. Eventually, these cells reach a point where irreversible changes in their degeneration cause cell death and exfoliation, which may make the cells unable to maintain fluid and ionic homeostasis [63,64,65], this may caused a marked shifting of extracellular water into the cells and thus lead to cellular swelling.

7. EFFECTS OF MALACHITE GREEN ON BEHAVIOURAL ASPECT OF FISHES

The fishes *Mystus vittatus* and *Anabas testudineus* exposed to malachite green at 96 hours LC_{50} dose, show a series of significant sequential behavioural responses at different duration of the treatment [66] (Singh &Singh, 2009). The sequence of these events is expedited with the increase in the concentration of the dye. In lower concentration, however, no significant behavioral response in marked. Roy (1988) reported that *Rita rita* (Bagrid catfish) shows first sign of restlessness followed by striking behavioral responses and ultimately death when exposed to the medium containing the detergent in the dose at which all fish die within 8-12 hours [67]. He further states that at 96 hours LC_{50} dose of detergent the fish don’t show any striking change in their behavior. This observation necessitates further experimentation as the fish treated with the dose at which they show 50% mortality should show symptoms of toxicity and changes in their behavior prior to their mortality. Garg & Mittal (1993) also observed changes in behavior of fish, *Clarius batrachus* (Walking catfish) exposed to lethal concentration of sodium dodecyl sulphate [68]. The stinging catfish, *Heteropneusts fossilis*, has been observed to exhibit alterations in behavior when exposed to trace elements, specifically zinc. Additionally, the fish has been observed to exhibit hyperactivity, which is typified by rapid pectoral and opercular movement, erratic swimming, and a gradual loss of equilibrium when exposed to the dye malachite green [36]. Brief periods of enhanced excitation after 1 hour of the dye treatment, followed by gradual increase in sluggishness and then signs of total loss of equilibrium and ataxia may be correlated as symptoms of toxicity caused by the malachite green affecting the CNS of the fishes. It is significant that in both the fishes- *Mystus vittatus* and *Anabas testudineus* exposed to malachite green, there is an increase in the surfacing activity than in control. They assume diagonal position with their slightly open mouth positioned just above the water level. This peculiar behavioral may be regarded as the sign of suffocation or asphyxia manifestation in the fish and may be related with the increased need to gulp air from the atmosphere for breathing. Srivastava *et al.* (1995a) have also reported breathing difficulties in the fish *Heteropneusts fossilis* under the influence of malachite green [36].

8. NUCLEAR ABNORMALITIES AND GENOTOXIC EFFECTS OF MALACHITE GREEN

Malachite green is genotoxic and carcinogenic dye [69,70,71] (Srivastava *et al.,* 2004; Bose *et al.,* 2005; Nebbia *et al.,* 2017), cytotoxic to mammalian cells [24] (Culp & Beland, 1996). It promotes liver tumours aggressively and causes malignant transformation [72] (Sandra *et al.,* 2006). Genotoxic effect revealed altered gene expression of CYP1A and HSP70 in *Crypinus carpio* on exposure to the MG for 15, 30 and 60 days [73] (Sinha *et al.,* 2021). Following dye exposure, the expression of HSP70, a significant oxidative stress indicator, rose. Several writers have documented fish micronuclei and nuclear anomalies caused by pesticides. In the peripheral RBC of *Channa punctatus* treated to chlorpirifos, Kumar S.P. (2012) found the existence of micronuclei and nuclear abnormalities such as notched, blebbed, and vacuolated nuclei [74]. Norman *et al.* (2008) observed significant increase of micronucleus in RBC of *Hypostomus plectomus* exposed to potassium dichromate dye [75]. Das *et. al.* (2014) observed presence of micronuclei in blood of spotted snakehead exposed to this triarylmethane dyes [76,77].

9. FUTURE PERSPECTIVE

Fisheries, a vital food source and global population supporter, must be carefully considered for potential threats to its economy and aquatic ecosystems. A coordinated effort should be made to evaluate the possible risks to the fish population, and stringent legislation should be implemented together with appropriate education initiatives for all parties involved to help them minimize health risks. In order to maintain the advancement of civilization, the scientific community should be given the full logistical assistance by the relevant authorities to search for any potential threats in the near future.

10. CONCLUSION

Malachite green has become one of the most talked-about and contentious dye used in aquaculture due to the concerns it presents to consumers, including impacts on the immune system and reproductive system as well as probable genotoxicity and carcinogenicity in some animals. A variety of aquatic and terrestrial creatures are severely poisoned by malachite green, which is very persistent in the environment. It presents possible environmental issues as well as significant risks to public health. Because malachite green is widely used as a fungicide in aquaculture practices and is also widely used in several industries, there is a high possibility of unintentional spillage of MG green in aquatic habitat. However, the high concentration, prolonged exposure, and high temperature of the aquatic habitat cause serious and hazardous biochemical and physiological changes that increase fish mortality and decrease fish fertility. The dye is still in use throughout the world despite being prohibited in a number of nations since there is insufficient knowledge about its harmful effects on the food chain and no suitable substitute. So the finding of proper alternative is very much essential and if it is used it should be used with proper guidelines (low concentration, used at low temperature, short exposure time) so that its deleterious effect should be avoided.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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