Potential Role of *Azolla pinnata* in Mitigating Abamectin Induced Hepatorenal Toxicity and Oxidative Stress in Nile tilapia, (*Oreochromis niloticus*)

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Abstract: The present study investigated the protective effect of *Azolla pinnata* 10 % as a dietary supplement in fish exposed to Abamectin (ABM). A bioassay experiment revealed that the median lethal concentration (LC_{50}) after 96 h was 0.06 mgL^{-1}. Four fish groups were used including the control group was fed commercial diet, *Azolla* group was fed a prepared diet of 10 % *Azolla* , ABM group treated with one-tenth 96h LC_{50} of ABM was fed commercial diet and the fourth group was ABM-*Azolla*, treated with one-tenth 96h-LC_{50} of ABM and fed a prepared diet of *Azolla* 10 %. The experiment lasted 28 days, during this period no mortality was observed. Results showed that ABM exposure triggered increase in the levels of serum alanine and aspartate aminotransferases (ALT and AST), urea, creatinine, and malondialdehyde (MDA). In contrast, the activity of the antioxidant enzyme catalase (CAT) and the level of reduced glutathione (GSH) declined. *Azolla* supplementation ameliorated the adverse effects of AMB and improved liver and kidney biomarkers. These biochemical effects were supported by histopathological examination of gills, liver and muscles. Therefore, *Azolla pinnata* could be used as a potential natural antioxidant to mitigate ABM toxicity in Nile tilapia.

Key words: Abamectin, *Azolla pinnata,* antioxidant, biochemical markers, Nile tilapia , Histological alterations.

INTRODUCTION

Pesticide are prevalent contaminants which cause different toxicological effects in living organisms and ecosystems (Tišler and Kožuh Eržen 2006; Khalil *et al.,* 2017; Galal *et al.,* 2018; Abu Zeid *et al.,* 2021; El-Bouhy *et al.,* 2021). Abamectin (ABM) is a widely used pesticide it belongs to the the avermectin group which are produced naturally by a soil actinomycete, *Streptomyces avermitilis* (Wislocki, *et al.,* 1989; Batiha *et al.,* 2020). It is a prominent active ingredient in various insecticides and nematicides. Furthermore, it is widely used in veterinary medicine, as a key pharmaceutical to protect against parasitic infections (Hedayati *et al.,* 2014; Vajargah *et al.,* 2021; Santos *et al.,* 2023). ABM residues may enter the aquatic environments through runoff and drift, causing deleterious effects to aquatic organism (Kolar *et al.,* 2008). ABM is lipophilic and degrades slowly so it can accumulate in different aquatic species, causing toxic effects (Yoon *et al.,* 2004; Novelli *et al.,* 2016; Santos *et al.,* 2023). ABM target glutamate-sensitive chloride channels causing disruption in neuronal coordination (Martin *et al.*, 2002; Yoon *et al.,* 2004; Gonzalez Canga *et al.,* 2009). This toxic effect is caused by the active compounds, avermectin B1a (80%) and B1b (20%), (Fisher and Mrozik 1989; Yoon *et al.,* 2004; Santos *et al.,* 2023). These neurotoxic effects can lead to liver and kidney dysfunction, alter blood parameters, and disrupt vital physiological processes (Qadir *et al.,* 2014; Kushwaha *et al.,* 2020; Salman *et al.,* 2022). The deleterious effects of ABM on fish highlighted the need for strategies to enhance fish health and resilience to contaminants.

A promising approach in this context is the use of natural dietary supplements in fish feed. Several studies reported that dietary supplements could improve nutrient absorption, boost immunity, and improve fish health, especially, in contaminated environments (Abd El-Gawad, and Abdel Hamid, 2014; Reda, and Selim, 2015; Selim, and Reda, 2015; Reda *et al.,* 2016; Mansour *et al.,* 2020; Rohmah *et al.,* 2022). Additionally, many studies have shown that some medicinal plants can protect fish from the harmful effects of pesticides in aquatic ecosystems, and enhancing their health and immunity (Hamed, and El-Sayed, 2019; Reda *et al.,* 2023).In this context, a promising aquatic plant called *Azolla pinnata* was proven to promote growth and immunity in fish (Basak *et al*., 2002; Majumder, 2022**;** Yohana *et al.,* 2023).

Azolla pinnata is a small floating aquatic fern; it is rich in protein, essential amino acids, vitamins and trace elements (Calder and kew 2002; Cherryl *et al*. 2014; Das *et al.,* 2018). Moreover, it has high content of carotenoid, alkaloids, flavonoids, phenols, tannins and saponins (Selvaraj *et al.,* 2013). Flavonoids, carotenoid and phenolic compounds in *Azolla* exhibit antioxidant, anti-inflammatory, and immune-modulating properties (Dhumal *et al.,* 2009; Tungmunnithum *et al.,* 2018). Additionally, *Azolla* contain essential macronutrients including, calcium, phosphorus, potassium, and other minerals (Anitha *et al.,* 2016). Because of its high nutritional value and antioxidant content, *Azolla* was proposed as an alternative medicine (Nayak *et al.,* 2015). Furthermore, *Azolla* protected Nile tilapia from the hepatorenal toxicity, oxidative stress and immunosuppression of imidacloprid (Attia *et al.,* 2021). Therefore, the current study aimed to investigate the ameliorative effect of *Azolla pinnata* on biochemical and histological changes in Nile tilapia exposed to sublethal concentration of ABM. These changes could be used as biomarkers for water contaminated with

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pesticides such as ABM. Enhancing fish resistance to ABM and other toxins through dietary supplementation could be a cost-effective and sustainable approach to mitigate the effects of pollution and improve fish health.

MATERIAL AND METHODS

Fish: Nile tilapias (*Oreochromis niloticus*) with initial weight of 35 ± 3 g were obtained from Aquaculture and Fish Technology Laboratory at Suez Canal University. Fish were kept in 60 L aquaria and acclimatized for two weeks. The aquaria were filled with dechlorinated tap water continuously ventilated by electric pumps. The temperature was maintained at 26 ± 1°C. During the acclimatization period, fish were fed with a commercial fish feed comprising 30% crude protein, 5% crude lipids, and 4% fiber twice daily. All experimental were conducted in accordance with the ethical standards set by the Ethics of Animal Use in Research Committee, Faculty of Agriculture, Suez Canal University, Egypt.

Diet preparation: Fresh *Azolla pinnata* were obtained from the Agricultural Research Centre. It was then dried and finely ground into powder. The experimental diets were prepared by grinding the *Azolla* powder with the commercial feed in portions of 10% *Azolla* and 90% commercial feed. Water was added to the mixture to make a paste. The paste was pelletized, air dried for 24 hours at room temperature. Thereafter, it was preserved at 4 °C until used.

Determining acute toxicity: Fish were distributed in 18 glass aquaria (10 fish /aquarium). Fish were exposed to commercial grade ABM (High keen® 5.4 % EC) at concentrations ranging from 50 to 250 μgL^{-1}. All concentrations were triplicated, and control group was kept in dechlorinated tap water without ABM. During the experimental period, fish were not fed, and the mortality rates were reported after 24, 48, 72, and 96 h. Dead fish were immediately removed from the aquaria. The LC_{50} value for 96 h and toxicological parameters were determined using Ld-P Line and Probit 2006 programs.

Experiment design: After the acclimatization period, 120 fish were randomly distributed into 12 glass aquaria with a capacity of 60 L (10 fish per aquarium, triplicates, 4 groups). Group 1, the control group was fed commercial feed. Group 2 was fed with the experimental diet containing 10% *Azolla*. Group 3 was exposed to ABM at one-tenth of the lethal concentration equals to 6 μgL^{-1} and was fed commercial feed. Group 4 was exposed to one-tenth of the lethal concentration of ABM and was fed with the experimental diet containing 10% *Azolla*. The experiment lasted for four weeks.

Evaluation of serum biochemical biomarkers:-

Sampling: The sampling procedure was as follows: The fish were handled and netted carefully to minimize stress. Using the clove oil at a concentration of 0.20 mL per 500 mL of water sedation was administered. Blood sample was obtained from the tail vein using a sterile disposable plastic syringe with a 22-gauge needle. The blood samples were subsequently placed in Eppendorf tubes and then centrifuged at 3000 rpm for 15 minutes. Subsequently, the serum was separated from the blood and stored at −20°C for subsequent serum assays, Fernandes *et al.,* (2017).

Biochemical analysis: Serum transaminases, alanine transaminase (ALT) and aspartate transaminase (AST), urea and creatinine were determined using commercial kits (Biodiagnostic Co., Giza, Egypt) according to the manufacturer instructions. The malondialdehyde (MDA) test measures lipid peroxidation, an indicator of oxidative stress in organisms, it was measured according to the method of Ohkawa *et al*. (1979). Glutathione (GSH) was measured by the methods of Beutler and Yeh (1963) the test is designed to measure the levels of reduced glutathione, an important antioxidant that helps protect cells from oxidative damage. Catalase (CAT) activity was measured by the methods of Aebi *et al.,* (1984).

Histological alterations: Five fish from each treatment were immersed in a solution containing 80 mg/L of clove oil after terminating the experiment. Subsequently, the animals were sacrificed, and their liver, gills and lateral muscle tissues were collected from all experimental groups. Bouin fixative solution was used to fix all tissues for a period of 48 hours. Thereafter, 50% ethanol was used to wash the fixed tissues, subsequently; a series of 60% and 90% absolute ethanol was used for dehydration, before clearing in xylene. Successively, the tissues were inserted in paraffin wax, and sections of 6 µm were made using a rotary microtome. The sections were stained with Mayer's hematoxylin and eosin (H&E) to examine malformation and differentiation of liver, gills, and muscles tissues. The stained slides were examined under a compound microscope, photographed and assessed.

Statistical analysis: Data are presented as means ± standard deviations. The LC_{50} value was calculated using Probit analysis and Ldp-line program based on Finney (1971). One-way analyses of variance and Student–Newman–Keuls method were used to compare results at $P>0.05$ (Sigma Stat software ver. 2.0, SPSS, Chicago, USA).

RESULTS AND DISCUSSIONS

Acute toxicity of ABM: Acute toxicity of ABM can be expressed as median lethal concentration (LC_{50}). In the current study, the LC_{50} after 96 h for ABM was 0.06 mgL^{-1} (Table 1). The LC_{25} and one-tenth of LC_{50} were also calculated (Table 1). The toxicity of ABM varies between different fish species, the 96-hour LC_{50} values in Zebra fish (*Danio rerio*) was 55.1 µg/L and 105.68 µg/L as reported by Tišler and Kožuh Eržen, (2006) and Santos *et al.*, (2023), respectively .In rainbow trout the 58 h LC_{50} was 3.4 µg/L (Jenčic *et al*., 2006), Wislocki *et al.,* (1989) reported that bluegill sunfish, sheephead minnow, and channel catfish were, 9.6, 15, and 24 µg/L, respectively (Wislocki *et al.,* 1989). These variations highlight the sensitivities of different fish species to ABM. The toxicity of ABM can be attributed to its ability to cross the blood brain barrier and bind to the GABA-gated chloride channels which make neurons unable to send signals resulting in paralysis and fish death (Martin, 1997., Jenčic *et al.*, 2006 and EL-Said, 2007).

Table (1): Acute toxicity parameters at 96 h in Nile tilapia

	Line equation Regression of probit(y) on log concentration(x)	Slope (b) Mean ± SE	LC_{50} mgL^{-1}	LC_{25} mgL^{-1}	1/10 of LC_{50} mgL^{-1}
ABM	**Y = 11.30 + 5 X**	5 ± 0.42	0.06	0.04	0.01

Hepatorenal markers: Table (2) shows the effect of *Azolla* 10%, ABM, and a combination of ABM and *Azolla* on liver and kidney function of Nile tilapia after 4 and 28 days. The activity of ALT increased significantly ($p \leq 0.05$) in fish exposed to ABM with percentages of 173.89% and 237.42% compared to the control after 4 and 28 days, respectively. Similarly, AST activity increased by 53.09% and 56.17% compared to the control group after 4 and 28 days, respectively. The increase in ALT and AST activities signifies the rapid destructive effect of ABM to liver cells even after the short period of exposure of 4 days.

Liver has a significant role in the metabolism and detoxification processes. ALT and AST are liver enzymes which play a crucial role in converting amino acids to energy, especially during periods of stress when the organism's energy demand increases. Changes in liver enzymes have been used as biomarkers of environmental contamination (Qadir *et al.,* 2014; Norhan *et al.,* 2022). In the current study, the hepatic enzymes ALT and AST increased in the ABM exposed group compared to the other experimental groups. In accordance to results reported in the current study, Rohmah *et al.,* (2022) reported a significant increase in serum ALT in Cyprinus carpio exposed to 12.5% LC_{50} of ABM for 30 days. Additionally, Fırat and Tutus (2020) reported increase in the activity of plasma ALT and AST in *O. niloticus* exposed to ABM for 96 h. Similarly, Hsu *et al.,* (2001) and Castanha Zanoli *et al.,* (2012) reported that ABM exposure increased the levels of these enzymes and damage hepatocytes. ABM may cause mitochondrial dysfunction resulting in hepatocyte toxicity (Castanha Zanoli *et al.,* 2012). ALT and AST are sensitive to hepatocyte damage and could be elevated in the absence of obvious symptoms. Consequently, measuring the activity of these enzymes can be useful to detect minimal cell damage (Coeurdacier *et al.,* 2011; Patriche *et al.,* 2011). This highlighted that ALT and AST can be used to monitor aquatic pollution. (Vaglio and Landriscina 1999; De la Torre *et al.,* 2000). Adding 10% *Azolla* to the diet of fish exposed to ABM resulted in a significant percentage decrease in the activity of ALT 16.2% at 4 days and 27.3% at 28 days compared to ABM alone. The same trend was observed for AST activity with a reduction of 10% at 4 days and 22.7% at 28 days, compared to ABM alone. This demonstrated that *Azolla* supplementation mitigated the deleterious impact of ABM on liver function in the ABM + *Azolla* group. The protective effect of *Azolla* could be attributed to its high content of polyphenols which have membrane stabilizing effects and act as antioxidants (Attia *et al.,* 2021).The role of dietary supplements to mitigate toxic effects of ABM was reported in other studies. Mansour *et al.,* (2022) reported that quercetin improved the toxic effects of ABM on Nile tilapia. Furthermore, Attia *et al.,* (2021) reported the role of *Azolla* to protect Nile tilapia from the hepatorenal toxicity, oxidative stress and immunosuppression of imidacloprid. Creatinine and urea are biochemical markers used to assess renal health (Gounden *et al.,* 2022). They are byproducts of protein metabolism; their levels could change due to the toxic effects of ABM on kidney (Nasr *et al.,* 2016). In the present study, urea levels in the ABM-treated group were elevated compared to control, with percentage increase of 58.31% after 4 days and 27.00% after 28 days. Creatinine levels showed dramatic changes, particularly in the ABM-treated groups, reflecting potential renal stress or damage. At 4 days, ABM led to a 337.50% increase, and at 28 days induced a 333.33% increase in creatinine levels compared to the control. These changes were highly significant ($p \leq 0.05$), indicating that ABM cause considerable stress on renal function. The percentage increase for both urea and creatinine levels was higher at 4 days compared to 28 days. This is likely due to acute renal stress and early metabolic disturbances following treatment with ABM. Over time, adaptation and compensatory mechanisms likely improved renal clearance of both urea and creatinine, resulting in a smaller increase by 28 days. In accordance with results reported in the current study, several studies reported elevation in serum urea levels after fish were exposed to ABM (5% EC) for 96 h (Farag and Reda; 2021), 20.73 µgL-1 ABM for 84 days (Mahmoud *et al.,* 2021) and ABM 103.68 µgL-1 after 14 days (El-Said, 2007). Kidney excretes urea, consequently, any increase in urea levels could result from reduction in glomerular filtration, dysfunction of tubules, and impaired kidney function. (Magdy *et al.* 2016; Farag and Reda 2021). After 4 days the combined ABM + *Azolla* treatment showed a 13.6% and 10.0% decrease in urea and creatinine levels, respectively, compared to ABM alone. These changes were highly significant ($p \leq 0.05$), indicating considerable stress on renal function, especially with ABM exposure. Urea levels were 7.1% lower in the combined treatment group compared to the ABM group after 28 days and creatinine level decreased by 51.9%. This significant reduction demonstrated that

Azolla alleviated the long-term impact of ABM on kidney function, particularly in terms of creatinine buildup, which is a marker of renal impairment. In earlier studies, *Azolla* improved liver and kidney function in Nile tilapia exposed to imidacloprid (Attia *et al.,* 2021) and improved resistance to bacterial infection in Nile tilapia (Mansour *et al.,* 2020). Similar protective effects were observed with other dietary supplements like S*pirulina platensis* in Nile tilapia exposed to diazinon (Abdelkhalek et al*.,* 2017) and *Moringa oleifera* in Nile tilapia exposed to pendimethalin (Hamed and El-Sayed, 2019) and fipronil (Mahmoud *et al*., 2022). **Oxidative stress markers:** The antioxidant enzymes CAT, MDA and GSH were assayed in the serum of Nile tilapia exposed to ABM and combination of ABM and *Azolla* Fig.1. Activity of the antioxidant enzymes in the control group represented the baseline (Fig.1). The *Azolla* 10% treatment group showed a slight insignificant decrease in CAT activity ($p > 0.05$), suggesting that *Azolla* alone did not affect CAT activity. In contrast, ABM induced a significant reduction in CAT activity ($p \leq 0.05$) compared to Control. This indicated that ABM caused oxidative stress and destroyed antioxidant defense mechanisms. Recovery of CAT activity was attenuated in the combination group of ABM + *Azolla* which was not significantly different from the Control group ($p > 0.05$), indicating that CAT activity returned to normal levels. MDA is a byproduct of lipid peroxidation, which is measured as a biomarker of oxidative stress and damage. ABM treatment resulted in significant elevation of MDA levels ($p \leq 0.05$) compared to the other treatments, indicating increased lipid peroxidation and oxidative damage (Fig. 1b). Nevertheless, Adding *Azolla* to the fish diet was effective in reducing MDA levels which was significantly lower ($p \leq 0.05$) than the ABM treated group (Fig. 1b). This suggested that *Azolla* has a protective effect and can reduce lipid peroxidation and oxidative stress induced by ABM.

Table (2): Effect of 1/10 LC_{50} of ABM and *Azolla* dietary supplementation on liver and kidney functions in Nile tilapia

Parameters	Time after treatment (days)	Treatments				*%Change
		Control	*Azolla* 10%	ABM	ABM -*Azolla*	
ALT activity	**4**	18.0 ± 1.70	20.6 ± 2.31	49.3 ± 1.53	41.3 ± 1.31	- 16.21
(U/L)	**28**	16.3 ± 1.60	19 ± 2.60	55.0 ± 0.50	40.0 ± 0.60	- 27.30
AST activity	**4**	72.7 ± 0.90	95.4 ± 1.51	111.3 ± 0.92	100 ± 1.81	-10.01
(U/L)	**28**	74.6 ± 0.90	89.2 ± 0.72	116.5 ± 1.51	90.0 ± 2.01	-22.70
Urea(BUN)	**4**	3.1 ± 0.01	4.0 ± 0.08	4.86 ± 0.55	4.20 ± 0.21	-13.60
(mg/dl)	**28**	2.0 ± 0.01	2.30 ± 0.30	2.54 ± 0.41	2.36 ± 0.05	-7.11
Creatinine	**4**	**0.2 ± 0.01**	**0.50 ± 0.05**	**0.71 ± 0.10**	**0.63 ± 0.06**	**-10.01**
(mg/dl)	**28**	**0.2 ± 0.03**	**0.40 ± 0.07**	**1.04 ± 0.02**	**0.50 ± 0.02**	**-51.90**

* %Change = [(ABM - *azolla*) – (ABM) / (ABM)] × 100, Values (means ± Standard divisions), (-) = decreasing percentage, ABM, abamectin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), ABM –*azolla*, the group that exposed to sublethal concentration of abamectin and fed on supplementary diet of *azolla* 10 %.

GSH is part of the defense system against oxidative stress. It helps in reducing harmful free radicals, reactive oxygen species and in detoxification of harmful chemicals. In the current study a significant decrease ($p \leq 0.05$) in GSH levels compared to the control was observed in ABM treated group (Fig. 1c). This suggested that ABM depletes antioxidant defenses by inducing oxidative stress. The levels of GSH were restored to control levels (Fig. 1c), indicating that *Azolla* helped to restore antioxidant defenses and recover GSH levels. The ameliorative effects of *Azolla*, which improved CAT antioxidant enzyme activity, increased GSH content, and reduced MDA levels in the ABM-*Azolla* group, agreed with the findings of Attia *et al.* (2021). Their study demonstrated that supplementing *Azolla* at 2% and 4% for eight weeks in Nile tilapia protected the liver and kidneys from imidacloprid toxicity. They reported enhancement in oxidative responses, serum biochemistry, and immune function. These results are also consistent with Mansour *et al.* (2020), who found that incorporating *Azolla* into fish diets enhanced antioxidant capacity, improved immunity, and increased survival rates, with slight improvements in growth performance.

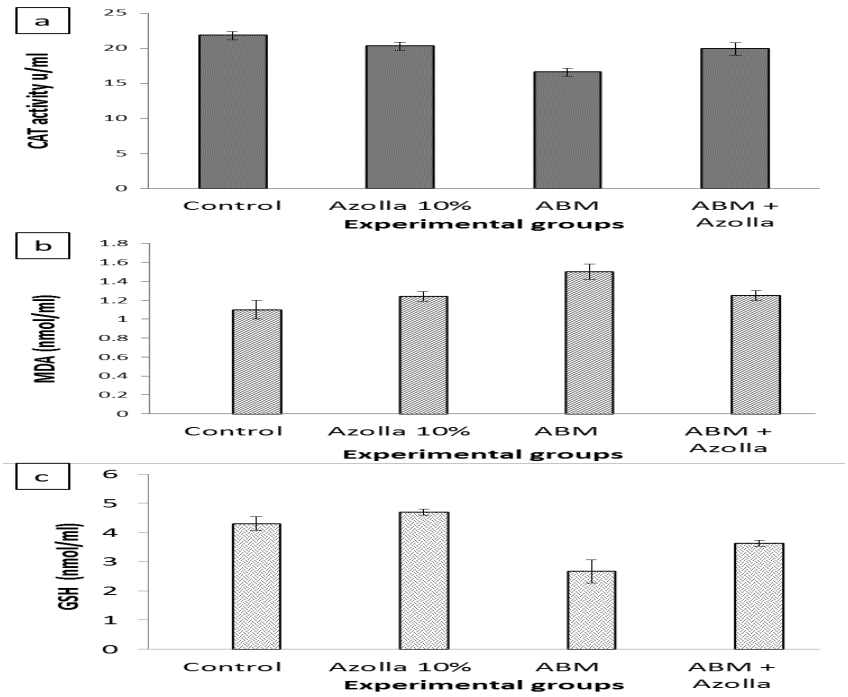

Fig. (1): Effect of ABM and dietary supplementation with *Azolla* on the antioxidant enzymes in the serum of Nile tilapia after 28 days. (a) CAT activity, (b) MDA and (c) GSH.

Histological alterations: To further demonstrate the efficacy of *Azolla* in reversing the sublethal effect of ABM and improve the hepatic and renal functions of Nile tilapia, histological alterations in the gill, liver and muscle tissues were studied. The sublethal effects of ABM on Nile tilapia gills were characterized by marked hyperplasia of inter-lamellar epithelia and congestion of the lamellar central veins of the gill filaments (Fig. 2 D). In contrast, gill filaments had a normal appearance in all other treatments (Fig. 2 A, B, C). In the liver, no histological alterations were observed in all treatments including control, *Azolla* and ABM-*Azolla* treated fish (Fig. 3 A, B, C). No vascular changes were observed, hepatocytes and cytoplasm were normal (Fig. 3 A, B, C). However, histological alterations were observed in the treatment of ABM, liver tissues showed infiltration of some melano-macrophage cells within the hepato-pancreatic acinus (Fig. 3D). Hepatocytes had finely granular cytoplasm around dilated hepatic sinusoids. Necrosis of hepatocytes in ABM-treated fish was observed after 28-day of exposure (Fig. 3 D). In ABM treated fish, muscle bundles showed huge infiltration of inflammatory cells within destructed bundles (Fig. 4 D). In contrast, no histological alterations were observed in all other treatments (Fig. 4 A, B, C). Histopathological alterations have recently been used as biomarkers to determine the pollutants effects on exposed organisms. Many investigations have used gills as a tool to monitor toxicity of many contaminants under laboratory conditions (Thophon, *et al.* 2003; Capkin, *et al.,* 2006; El Said, 2007; Florez-Lopes, and Thomas, 2011; Thanomsit, *et al.* 2016; Hendawy *et al.,* 2024). In this study, the observed alterations of gills in fish exposed to ABM agreed with the study of El-Said (2007) who observed necrosis and deformation of gills lamellae in fish exposed to sublethal concentration of ABM. The observed alterations in gill filaments depend on toxicant concentration and exposure time (Thanomsit *et al.,* 2016). Various alterations may be due to the expression of defense mechanisms against different pollutants. The changes of gills included separation of epithelium cells from lamellae and lamellar edema of the gills. The gills are in intimate contact with the surrounding environment and are also responsive to alterations in water quality, making them a primary organ for the deposition of the contaminants, (Osman, *et al.* 2010). Liver is the main target organ for studying the toxicity of pollutants as it responsible for detoxification and bioaccumulation of toxins (Mohamed, 2009). In accordance with results from this study, several liver alterations in fish exposed to pesticides were reported in earlier studies including, vacuolation, blood congestion, necrosis of hepatocytes, several changes of microvilli and enlargement of sinusoid, (Alkaladi, *et al.,* 2013 and 2014; Hasan *et al*. 2015; Thanomsit *et al.,* 2016; Yancheva *et al*.,2016; Kushwaha *et al.,* 2020; Hendawy *et al.,* 2024). In this study muscle bundles showed huge infiltration of inflammatory cells within destructed bundles in ABM treated fish which agreed with Hendawy *et al.,* (2024), who observed minimal interstitial edema and morpho-histological examination of muscle fibers in fish exposed to ABM. Thus, this study elucidated the potential role of *Azolla* in avoiding the deleterious side effects of ABM in Nile tilapia. These side effects included biochemical changes in fish serum AST, ALT, urea, creatinine, CAT, MDA and GSH. Furthermore, the use of *Azolla* helped in recovering histopathological alternation in gill, liver and muscles of tilapia fish. The studied biochemical and histopathological alternations could be used as biomarkers for water pollution with insecticides.

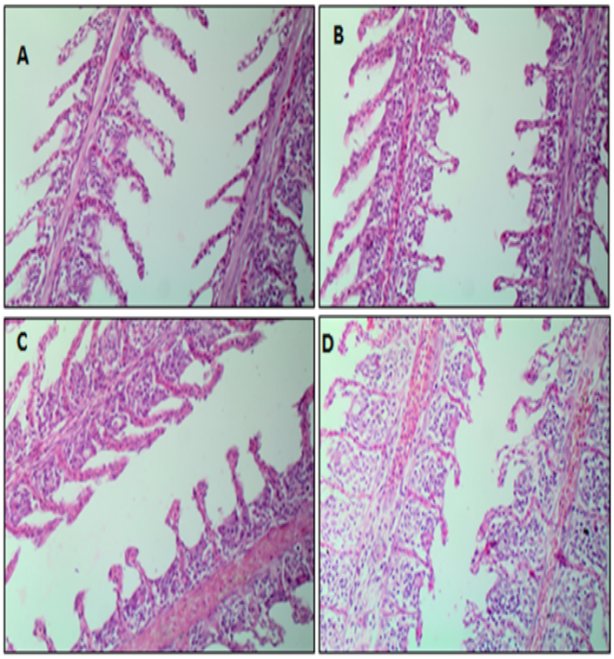

Fig (2): Light micrographs of the gill of Nile tilapia showing normal gill for A, control, B, *Azolla* treatment and C, ABM-*Azolla* treatment while D refers to gill filaments of ABM treated fish. Gill filaments showing marked hyperplasia of inter-lamellar epithelia, congestion of lamellar central veins and lifting of lamellar lining epithelia.

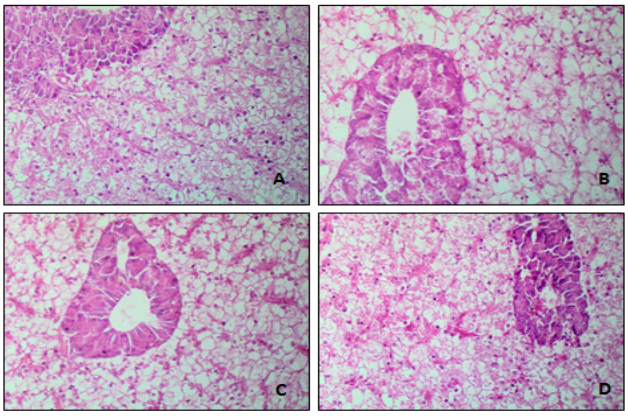

Fig. (3): Light micrographs of the liver of tilapia fish showing normal liver of A, control, B, *Azolla* treatment, C, ABM-*Azolla* treatment and ABM- treated fish (D) showing infiltration of some melano-macrophage cells within the hepato-pancreatic acinus. Hepatocytes have finely granular cytoplasm around dilated hepatic sinusoids.

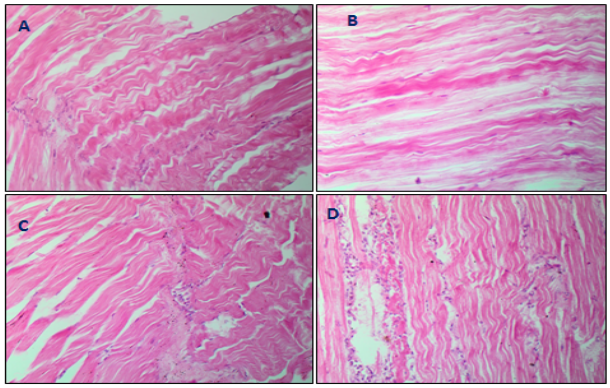

Fig. (4): Light micrographs of the liver of tilapia fish showing normal liver of A, control, B, *Azolla* treatment, C, ABM-*Azolla* treatment, and ABM- treated fish (D).

CONCLUSION

The current investigation demonstrated the promising role of *Azolla pinnata* as a potential natural detoxifying agent. The supplementation of *Azolla* at 10% for 28 days enhanced the oxidative responses in Nile tilapia exposed to ABM at sublethal concentration. This was evidenced by the restoration of CAT activity, increased GSH levels, and reduced MDA levels, compared to ABM treatment alone. Furthermore, dietary supplementation of *Azolla* restored the liver and kidney function, protected gills, liver and muscle tissues and reversed the damage caused by ABM. Therefore, *Azolla* can be used as a natural intervention to enhance antioxidant capacity and protect aquatic organisms from the harmful effects of environmental contaminants. Further research is recommended to explore the long-term efficacy and broader applicability of *Azolla* in mitigating oxidative stress and enhancing health outcomes.

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الدور المحتمل للأزولا بيناتا في التخفيف من السمية الكبدية الكلوية والأكسدة المستحثة بالأبامكتين في أسماك البلطي النيلي ((*Oreochromis niloticus*

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المستخلص: بحثت هذه الدراسة في التأثير الوقائي للأزولا بيناتا باستخدامها كمكمل غذائى وبخلطة مع الغذاء الاساسى للاسماك بنسبة 10 % وحماية الأسماك المعرضة لتركيزات تحت المميتة من مبيد الأبامكتين (ABM). كشفت تجربة التقييم الحيوى أن متوسط التركيز المميت (LC_{50})بعد 96 ساعة من مبيد الابامكتين على اسماك البلطى النيلى، كان (0.06 ملجم لتر$^{-1}$)، ثم استخدمت أربعة مجموعات سمكية لدراسة تأثير مكملات الأزولا الغذائية. المجموعة الضابطة تم تغذيتها بنظام غذائي تجاري، ومجموعة الأزولا تم تغذيتها بنظام غذائي محضر من الأزولا بنسبة 10% من الأزولا و90 % من الغذاء التجارى، ومجموعة ABM المعالجة بعشر التركيز المميت ل 50 % من الافراد المعاملة بعد 96 ساعة- والتى تم تغذيتها على نظام غذائي محضر مع الازولا بنسبة 10% ، والمجموعة الرابعة كانت مجموعة-ABM تم تغذيتها بنظام غذائي تجارى. استمرت التجربة 28 يومًا، وخلال هذه الفترة لم تلاحظ أي نسبة موت فى مجاميع الاسماك المعاملة. أظهرت النتائج ارتفاعاً في مستويات إنزيمات الألانين والأسبارتات أمينوترانسفيراز (ALT) و (AST) في المصل واليوريا والكرياتينين والمالونديالديهيد(MDA) ، وفي الوقت نفسه، انخفض نشاط الإنزيمات المضادة للأكسدة (CAT) ومحتوى الجلوتاثيون المختزل (GSH) بشكل ملحوظ في المجموعة التي تعرضت للأبامكتين. قللت مكملات الأزولا بشكل ملحوظ من هذه المتغيرات وحسّنت علامات الكبد والكلى. ولذلك، يظهراستخدام الأزولا بيناتا كمكمل غذائى للاسماك إمكانية استخدامه كمضاد طبيعي للأكسدة للتخفيف من سمية الأبامكتين في أسماك البلطي النيلي.