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**ATTENUATING EFFECTS OF GREEN TEA AND GARLIC EXTRACT IN  
CYPERMETHRIN POISONED FISHES, *Heteropneustes fossilis*: A REVIEW**

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**ABSTRACT**

Cypermethrin used throughout the world in the agricultural fields and domestic purposes to control pests is known to have toxic potential to non-target aquatic organisms specially fishes. Poisoning leads to disturbance in the fish metabolism and other physiological process causing mortality in fishes. Flavonoids are the polyphenolic compounds present in green tea and garlic extract having various beneficial effects related to low toxicity in fishes. Besides several advantageous effects flavonoids also possess antioxidant property reducing oxidative damage caused by cypermethrin. The major polyphenols present in green tea are epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate (EGCG) in which EGCG is the most active component. Garlic consists of sulfur and non sulfur compounds like alliin, ajoene, diallyl polysulfides and S-allylcysteine in which the most active component of garlic is alliin (S-allyl cysteine sulfoxide) which is a potent antioxidant reducing non-target exposure of hazardous pesticides. The aim of this review is to demonstrate the adverse effects of cypermethrin in fishes and its mitigation by flavonoids (GTE and GE). Cypermethrin altered various biochemical parameters especially liver enzymes and kidney markers in fishes following exposure. The combination therapy i.e. cypermethrin along with GTE and GE combats cypermethrin toxicity.

**Keywords: Polyphenols, Green tea, Garlic, Cypermethrin, Fishes**

## INTRODUCTION

The use of pesticides in agriculture throughout the world has become necessary for the protection of the plants against insects, plant pathogens, pests, weeds and diseases to obtain higher productivity to fulfill the food requirement of increasing population. Apart from their significant contribution in the pests control, the pesticides, on the other hand, have resulted in contamination of agro-ecosystem which cause heavy losses to the environment and may have adverse effects on non target organisms and also their remains in the environment lead to serious public health concerns [1, 2]. Different pesticides are being recommended in India include organochloride, organophosphate, pyrethroids, carbamate and some biopesticides due to their higher efficacy against the pests of crops. The pesticides continuously added to water bodies throw domestic sewage, industrial waste and from agricultural fields show negative effects on non-targeted organisms particularly fishes and other aquatic organisms [3, 4].

## CYPERMETHRIN TOXICITY

Cypermethrin is one of the synthetic pyrethroid used worldwide. Different studies showed that cypermethrin generate adverse effects on the non target organisms including

both vertebrates and invertebrates [5, 6]. Cypermethrin contamination is dangerous to aquatic environment specially fishes. Toxicity studies of cypermethrin on fishes were done by many researchers [7, 8].

### Acute toxicity

Acute toxicity is the extreme effect suffered by an organism from short term exposure to toxic chemicals. Acute toxicity testing is a basic necessity for toxicity studies. The data obtained from acute toxicity are important and beneficial in the fixation of sub-lethal concentrations for chronic toxicity tests. Several workers obtained LC<sub>50</sub> values during acute toxicity bioassay of cypermethrin on different species of fishes [9, 10]. The LC<sub>50</sub> value obtained from acute toxicity of cypermethrin to fresh water catfish *Heteropneustes fossilis* by Saha and Kaviraj (2003) was 0.67 µg/L after 72 hr exposure [7]. Sarikaya (2009) investigated acute toxicity on adult Nile Tilapia (*Oreochromis niloticus*), whose estimated 96 hr LC<sub>50</sub> value of cypermethrin was 5.99 µg/L [11]. Study on cypermethrin in fresh water fishes *Channa punctatus* in bundelkhand region was carried out by Vishwakarma and Zahra, (2009). Investigation was done to determine the toxic effect on mortality of highly active pyrethroid insecticides, cypermethrin, 4% EC

(Profex Super), 10% EC (Dhanuka Super Killer-10), 25% EC (Sunthrin-25). The results showed that toxicity was found to be increased with the proportion to concentrations of toxicants following 24-96 hr exposure [12]. Singh *et al.*, (2010) observed toxicological alterations of cypermethrin exposure (24 or 96hr) against freshwater teleost *Colisa fasciatus* in dose and time dependant manner at different seasons and found decreased LC<sub>50</sub> value from 0.009(24hr) to 0.006(96hr) in winters and 0.06(24hr) to 0.02(96hr) mg/L during summer season. Their study also showed that a sub-lethal dose of cypermethrin alters the levels of various biochemical parameters [13]. Tiwari *et al.*, (2012) investigated the toxicity of cypermethrin at very low concentration in fingerlings of *Labeo rohita*, the LC<sub>50</sub> values represent decreasing trend as 0.323 µg/L (6 hr) > 0.278 µg/L (12 hr), > 0.240 µg/L(18 hr) and > 0.205 µg/L (24hr) on increasing exposure period from 6-24hr [14]. The response of acute toxicity following cypermethrin exposure for 96 hr on biochemical parameters of juveniles of *Clarius gariepinus* was studied by Ojutiku *et al.*,(2013) , the different values of cypermthrin used during the experiments was 0.025 mg/L, 0.050 mg/L, 0.075 mg/L, 0.100 mg/L and 0.125 mg/L [15]. Kannan *et al.*,

(2014) worked on different hematological and biochemical parameters in *Catla catla* at the LC<sub>50</sub> of cypermethrin (0.0006 mL/L) for 24hr and reported an increase in glucose and decrease in total proteins level in blood of exposed fish compared to control group [16]. Orun *et al.*, (2014) conducted biochemical studies on *Oncorhynchus mykiss* intoxicated by 3 concentrations of cypermethrin for 96 hr viz. 0.0123, 0.0082 and 0.0041 ppm. The LC<sub>50</sub> values obtained in our investigation that is 0.00066, 0.00044, 0.00033 and 0.00022 mL/L after 24, 48, 72 and 96 hr respectively in *Heteropneustes fossilis* are in agreement with the results of the above workers [17]. Majumder and Kaviraj, 2017 conducted experimental work on cypermethrin toxicated stress and changes in growth of fish *Oreochromis niloticus* for 14-28 days shows alteration in several enzymes such as reduction of hepatic glycogen, ALP, catalase and elevation in glucose level, phosphatase AST and ALT in liver [18]. In the present investigation the elevated levels of serum SGOT, SGPT, ALP, ACP, uric acid, creatinine and blood glucose were observed while decline was seen in serum total protein and tissue glycogen level after acute and chronic exposure of cypermethrin in *Heteropneustes fossilis*.

### Biochemical parameters

Pesticide exposure causes several changes in the biochemistry of fishes. Various literatures are available regarding the effects of cypermethrin on haematological and biochemical parameters in serum and histological characteristics in different tissues of fishes [8, 19-22]. The toxicity bioassay test of synthetic pyrethroid insecticide cypermethrin was conducted by Das and Mukherjee (2003), they were reported sublethal concentration of cypermethrin (1/10 and 1/50 of 96 hr LC<sub>50</sub>) on biochemical parameters in blood and different organs of carp *labeo rohita*. Significant reduction was found in serum protein and ALP but ACP level remains unchanged and blood glucose was found to be elevated in treated groups [23]. Sarkar *et al.*, (2005) examined histopathological alterations in the liver of *Labeo rohita* after chronic exposure (28 days) of carbofuran and cypermethrin, in which major damages includes diffuse necrosis, individualization of hepatocytes, hyperplasia, disintegration of hepatic mass etc. Results also showed prominent recovery when fishes were kept in fresh water for additional 28 days without toxicants but faster recovery occurred in carbofuran than cypermethrin [19]. Begum (2007) studied the effect of cypermethrin on biochemical

parameters like protein, AST and ALT enzymes in liver, muscle, gill and kidney tissues of the fish *Clarias batrachus*. The fishes were exposed at concentration of 0.07 mg/L of cypermethrin for 10 days in which increased enzyme activities were observed in all the tissues and decreased level of protein was observed in muscle and kidney tissue [24]. Singh *et al.*, (2010) investigated biochemical alterations of cypermethrin against freshwater teleost fish *Colisa fasciatus* in different season, their results showed altered level of total protein and free amino acid in muscle and liver tissues by the sub-lethal doses of cypermethrin after 96hr [13]. Tantarapale (2011) examined the impact of cypermethrin on total protein content in liver and muscle tissues of the freshwater fish *Channa striatus*, the reduction of total protein content was found more in muscle than in liver tissues at different exposure period (24-96 hr) of the cypermethrin [25]. Kannan *et al.*, (2014) studied the effects of cypermethrin (10% EC) on *Catla catla* in the concentration of 0.0006 ml/l for 24hr and reported an increase in glucose and decrease in total protein level. Bhanu and Deepak, (2015) determined the impact of cypermethrin on serum biochemical parameters in freshwater fish *Cyprinus carpio*. The fishes were reared in sublethal

concentration of cypermethrin (0.2 ppm) for 28 days. The study revealed that the glucose and creatinine rise in the blood whereas the protein level depletes significantly [26].

Certain studies were also reported regarding the toxic effects of cypermethrin in *Heteropneustes fossilis*. Mishra *et al.*, (2001) recorded the altered level of plasma calcium and inorganic phosphate and behavioral responses in *Heteropneustes fossilis* in response to cypermethrin treatment [27]. Similarly, Pandey *et al.*, (2009) conducted study to determine the toxicity of cypermethrin and its effects on serum electrolytes ( $\text{Ca}_{+2}$ ,  $\text{Mg}_{+2}$  and  $\text{P}_i$ ) for a period of 96 hr (3.42  $\mu\text{g/L}$   $\text{LC}_{50}$ ) and recovery response in catfish *Heteropneustes fossilis*. The level of calcium decreased and  $\text{Mg}_{+2}$  and  $\text{P}_i$  increased in the exposed fishes up to 96 hr. To study the recovery response fishes were kept in toxicant free water for another 96 hr. During recovery period the level of serum electrolytes observed a pattern towards normalcy when compared with 96 hr treated fishes [28]. Joshi *et al.*, (2007) reported cypermethrin induced histopathological alterations in the liver of *Heteropneustes fossilis* in chronic toxicity experiments ( $\frac{1}{4}$  of 96 hr  $\text{LC}_{50}$ ) to determine tissue damage. Observed histopathological changes in liver include necrosis and fibrosis at different

periods of exposure (20, 30, 40 and 60 days) [29]. Saha and Kaviraj, (2009) investigated cypermethrin toxicity in freshwater catfish *Heteropneustes fossilis*. Two sub-lethal concentrations (0.3 and 0.5  $\mu\text{g/L}$ ) of cypermethrin were taken and results recorded as rise in plasma glucose level and reduction in the liver glycogen level after 4 hr of toxicant exposure. At the same time activities of acid and alkaline phosphatase of liver decreased significantly as compared to control [30]. Deka and Dutta, 2012 investigated cypermethrin impacts on some haematological parameters (TEC and Hb content) in cat fishes (*Heteropneustes fossilis*). The obtained 96hr  $\text{LC}_{50}$  value of cypermethrin was 0.67 $\mu\text{g/L}$ . Fishes were introduced to 0.1  $\mu\text{g/L}$  ( $\frac{1}{6}$ <sup>th</sup> of 96hr  $\text{LC}_{50}$  of cypermethrin for 24, 48, 72 and 96 hr). A significant decrease in the TEC and Hb content was observed after treatment of cypermethrin when compared to control [22]. Impact of cypermethrin on the level of blood glucose and urea in *Heteropneustes fossilis* was studied by Deka and Dutta, 2015. Fishes were introduced to sub-lethal concentration of cypermethrin (0.96  $\mu\text{g/L}$ ) for 24, 48, 72 and 96 hr. A significant increase in the blood glucose and urea levels were observed in treated groups compared to non- treated groups [8]. The  $\text{LC}_{50}$  of cypermethrin

evaluated in present study was found to be 0.00066 mL/L, 0.00044 mL/L, 0.00033 mL/L and 0.00022 mL/L after 24, 48, 72 and 96 hr exposure in *Heteropneustes fossilis*. After acute and chronic exposure increased in the levels of serum SGOT, SGPT, ALP, ACP, Creatinine, Uric acid and Blood glucose while decreased activity was observed in serum total protein and tissue glycogen.

### FLAVONOIDS

Flavonoid, today is one of the active constituent contributed to the progress in advancing the knowledge of plants derivatives in the field of research. The present literature survey deals with the general features of the flavonoids in the development of new strategy for improved defense response in various experimental models. Flavonoids belongs to a group of natural substances with different phenolic structures which are present in fruits, vegetables, roots, stems, bark, flowers, grains, tea, and wine [31]. The best known function of almost every group of flavonoids is that, their capacity to act as antioxidants scavenging reactive oxygen species [32]. Later, many other functions have been attributed to flavonoids like anti-inflammatory, antiallergic, antiviral, antibacterial, anti-thrombogenic, and anti-

carcinogenic properties [33-37]. Numerous studies have been carried out on protective effects of flavonoids against pesticide toxicity in mammals [38, 39].

### Green tea

Green tea is the most popularly consumed beverage in all over the world which is obtained from the leaves of the plant *Camellia Sinesis*. Polyphenols are the major components of green tea which are responsible for the potential pharmacokinetic properties and other beneficial effects [40, 41]. The polyphenols present in green tea is catechins and the major catechins present in green tea are - epicatechin (EC), epigallocatechin gallate (EGCG), epigallocatechins (EGC) and epicatechin gallate (ECG) which have higher antioxidant potentials. The strong antioxidant property of green tea makes it more effective in protecting the body from oxidative damage caused by free radicals. The role of green tea on fishes was done by many researchers [42, 43]. Cho *et al.*, (2007) reported effect of dietary sources of green tea on growth, blood chemistry and body composition of the juvenile olive flounder (*Paralichthys olivaceus*). Specific growth rate and weight gain in fishes was recorded which were fed with green tea containing diet. Higher crude lipid content, LDL and lower GPT level were

estimated in fishes fed with control diet than that fed with the diet containing green tea sources [44]. Yadav *et al.*, (2015) demonstrated impact of green tea extract on the cypermethrin induced toxicity on tissues of liver and muscles in fresh water fishes, *Heteropneustes fossilis*. Results showed decrease in the protein concentration of liver and muscle, but due to supplementation of flavonoid protein concentration was found to be increased [45]. Zahra *et al.*, (2016) demonstrated the mitigating effect of green tea extract on acute toxicity of cypermethrin in fresh water fishes, *Channa punctatus*. Estimated LC<sub>50</sub> values were 0.00087mL/L, 0.00079mL/L, 0.00065mL/L and 0.00050mL/L after 24, 48, 72 and 96 hr exposure respectively. The mortality rate was found to be reduced after addition of green tea extract, which shows that GTE suppresses the effect of pesticides [46]. Al-Ngada *et al.*, (2017) performed the effect of dietary supplementation of green tea (*Camellia Sinesis*) on growth, body composition and serum biochemistry of the Asian Seabass, (*Lates calcarifer*) fingerlings after 90 days. Results showed a significant improvement of growth performance in all the fishes which were fed with green tea (10 g/kg<sup>-1</sup> and 20 g/kg<sup>-1</sup>) as additive when compared with control, while the maximum performance

was achieved from the treatment with lower dose (10 g/kg<sup>-1</sup>) of green tea. The study concluded that, the diet with low levels of green tea as food additives has improved the growth performance and serum biochemical parameters (glucose, total protein, ALT, AST, ALP, uric acid) [47]. The alleviation by green tea on growth, blood and immune parameters in Sturgeon hybrid (*Huso huso x Acipenser ruthenus*) after 6 weeks was investigated by Hasanpour *et al.*, (2017). Feeding with oxidized fish oil had no effects on growth rate however feed along with green tea extract provided improved growth rate. In blood chemistry only WBC numbers became increased on receiving feed with green tea. Certain immune parameters were found to increase in green tea added diet also [48]. Hwang *et al.*, (2013) also found that green tea extract improves growth rate, body composition, and recovery against stress in the juvenile black rockfish (*Sebastes schlegeli*) [49]. In our results after addition of GTE mortality reduces gradually in low concentration of GTE which is due to antioxidant property of green tea which normalizes the toxic effect of cypermethrin. The effective concentration of green tea was found to be 0.5 mL/L at which no mortality was occurred in fishes. After addition of GTE all the parameters tends to become

normalized. Hence GTE restored the damage caused by cypermethrin in fishes.

### Garlic extract

In the view of the role of many dietary constituents obtained from plants to detoxifying enzyme system, attention has been focused on their protective role. Garlic has been of much interest among them and its consumption has increased due to the presence of some active constituents associated with health benefits [50]. There are numerous observations and a number of laboratory studies which indicate that garlic have protective effects on fishes [51, 52]. Study on assessment of garlic extract on some hematological parameters, growth and disease resistance against *Aeromonas hydrophila* in African catfish *clarias gariepinus* was investigated by Thanikachalam *et al.*, (2010). Powder of garlic peel (0%, 0.5%, 1.0% and 1.5%) was added into the diet and provided to fingerlings for 20 days. Biochemical parameters (serum albumin, globulin and total protein) and hematological parameters (WBCs and RBCs) were examined. Enhanced albumin, globulin and serum protein content was estimated in fishes fed with dosages of garlic peel to control group. Significantly increased WBCs and RBCs counts were observed in garlic peel contained

diet groups to control group. Lower survival rate in control groups and higher survival rates were found in the garlic peel diet groups against *A. hydrophila* [53]. Fazlolahzadeh *et al.*, (2011) studied effect of garlic on serum AST, ALT and some haematological parameters (MCV, MCH, MCHC) when *Rainbow trout* is under heat stressed condition. The altered level of AST, ALT, MCV, MCH and MCHC was seen in all treatments when compared to control and suitable doses of garlic are able to balanced all parameters [54]. Ndong and Fall, (2011) discussed about the ameliorative property of garlic on growth and immune responses in hybrid tilapia (*oreochromis niloticus x oreochromis aureus*) [55]. Naeiji *et al.*, (2013) investigated the effects of dietary garlic extract on serum biochemical parameters, protein oxidation and lipid peroxidation in the tissues in common carp *Cyprinus carpio*. Dietary garlic powder (25 and 50 g/kg feed) was supplied for 6 weeks to fishes. A significant decrease in MDA level of liver and kidney was observed at garlic diet with 50 g/kg while non significant decrease in MDA level at 25 g/kg garlic diet. Protein carbonyl contents and AST activity were decreased significantly in muscle after garlic administration at 25 g/kg diet. Whereas decreased ALP activity was observed in both

the doses. There was no significant effect on total protein, albumin, creatinine, and gamma-glutamyl transferase activity after garlic supplementation. The results indicated that garlic has potential to decrease oxidative stress [56]. A comparative study on the antioxidant and antimicrobial effects of garlic and ginger (*Zingiber officinale*) on physiochemical and microbiological characteristics of liquid smoked silver carp *Hypophthalmichthys molitrix* during preservation was done by Frank Fijelu *et al.*, (2014) and their study suggested that both ginger and garlic have antioxidant and antimicrobial potential during storage [57]. Antagonistic efficiency of dietary allicin in reducing deltamethrin- induced oxidative damage in Nile tilapia (*Oreochromis niloticus*) was investigated by Abdel-Daim *et al.*, (2015). After treatment of deltamethrin increased serum ALT, AST, ALP, urea, uric acid, cholesterol, creatinine and tissue MDA content and decreased serum total protein and albumin and lipid peroxidation enzymes (GSH, GSH-Px, SOD & CAT) in tissues were noticed. Garlic supplementation enhanced the altered serum biochemical parameters and antioxidant and lipid peroxidation biomarkers [39]. Rodge *et al.*, (2018) studied the garlic induced changes on serum biochemical and hematological profile

of *Clarias batrachus* when exposed for 21 days. Results revealed that garlic improved hematological composition, protein, albumin, creatinine and lipid profile content of serum [58]. El-Sokkary *et al.*, (2018) conducted study on the impact of green tea and garlic on the morphological alterations and oxidative stress damage in aged male and female rats. Administration of green tea and garlic improves the histological appearance in the cells and found protection against oxidative stress parameters (LPO, SOD and GSH) in male rats than female [59]. In our results the mortality rate in fishes decreased after all exposure periods and 10mL/L of garlic extract was found to be effective at which no mortality observed in fishes as they received LC<sub>50</sub> concentration of cypermethrin. This shows the vital role of garlic as an antioxidant which increases the survival rate of fishes intoxicated by cypermethrin.

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