

BIOCHEMICAL ALTERATIONS EFFECTING CARBOHYDRATE METABOLISM OF *CYPRINUS CARPIO* (COMMON CARP) ON EXPOSURE TO BOTANICAL PESTICIDE – ANOSOM.**¹Syeda Hina Kauser and ¹Rafath Yasmeen**¹Department of Zoology, University College of Science, Osmania University, Hyderabad, Telangana State, 500007, India.*Corresponding Author. Email: hina_kauser2003@yahoo.com**ABSTRACT**

The effect of biopesticide - Anosom on certain metabolites and enzymes of carbohydrate metabolism were assessed in the gill, liver, kidney and muscle of *Cyprinus carpio* during sub-lethal toxicity exposure for the period of 14 days. A dose of 0.01 ppm was taken as the sub-lethal dose after determination of Lc50 value. The tissues were taken from exposed and control fish at the end of 24 hrs., 7days, 14 days and used for the estimation of total carbohydrates, total glycogen, succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities. All the organs showed the significant difference between control and exposed groups in all the estimated parameters throughout the days of exposure. In the present study, all the investigations i.e., total carbohydrates, total glycogen, SDH and LDH showed significant decrease as the days of sub-lethal exposure increased, up to the completion of 14 days of exposure. The present study considers biochemical parameters as important biomarkers in determining the level of toxicity caused by the biopesticide – Anosom.

KEYWORDS: aquatic ecosystems, *Cyprinus carpio*, pesticides.**INTRODUCTION**

Contamination of the water bodies has become a major problem at local, regional, national, and global levels (Spalding *et al.*, 2003). The water bodies contain a large number of pollutants such as chemical compounds, industrial and agricultural wastes. Insecticides constitute the major pollutants of many aquatic habitats. The major routes of insecticides polluting the aquatic ecosystems include rainfall, runoff, and atmospheric deposition. The insecticides finally make their way into ponds, lakes, and rivers (Arjmandi *et al.*, 2010) and exhibit some kind of toxic effects on non-target organisms. Presence of toxic substances such as pesticides, in the aquatic environment causes a reduction in the quality of water which leads to health hazards in the aquatic organisms especially fish (Roberts, 2001).

The contamination of aquatic ecosystems by pesticides causes harmful effects on health, growth, survival and reproduction of aquatic animals especially fishes, which constitute an important source of food for human and animal consumption (Banaee *et al.*, 2008). Fishes are extremely sensitive to any kind of pollutants present in the water. Hence, pesticides may cause significant alterations in certain biochemical processes in the tissues of fish (John, 2007). It has also been found that the pesticides can cause serious alterations in the physiological well-being and health condition of fishes (Begum, 2004). Since fishes are the important food source for humans, therefore the health of fish is important.

The biochemical alterations in organisms are considered as most sensitive and earliest events of any pollutant damage. Effects of pesticides on biochemical processes in aquatic animals have been done earlier in India by Tripathi and Singh (2002). The metabolites of carbohydrate metabolism and enzymes examined are one of the most important parts of biological function. Similar kind of work i.e., the effect of pesticides on fishes has also been done by Begum (2004). Exposure to pesticides causes severe alterations in tissue biochemistry of fishes (Shrivastava and Singh, 2004). Hence, biochemical parameters are the best physiological indicators of the fish health. Therefore they are important to be focused while studying the toxic effects of various pesticides and pollutants on fish.

The pesticide used in the present work is a commercially available plant-based biopesticide Anosom. The fish species used for the study is the common carp *Cyprinus carpio*. Common carp is a very popular edible fish and is

grown in rice fields in some states of India as a practice of integrated farming. It is grown in many natural and cultured in artificial ponds because of its great commercial value. The aim of the present study is to estimate the biochemical changes of carbohydrate metabolism and related enzymes in various tissues of *C. carpio* during sub-lethal exposure to Anosom.

MATERIALS AND METHODS

C. carpio ranging in length 14 ± 0.95 cm and weighing 28.59 ± 1.80 g were collected from Kaikaluru village of Andhra Pradesh State and transported to the laboratory in well-aerated condition. The fish were acclimatized in well-aerated tanks for a period of one month. They were fed twice daily with commercially available fish feed pellets and the water was renewed daily. The 96 hrs LC50 value of Anosom was already estimated as 0.1 ppm. $1/10^{\text{th}}$ of the 96 hrs LC50 value i.e., 0.01 ppm is taken as the sub-lethal dose. The fish were exposed to the sub-lethal dose for a period of 14 days. After the completion of 24 hrs, 7 days and 14 days the fish of both control and exposed groups were dissected and the organs (gill, liver, kidney and muscle) from six individuals were collected and estimated for biochemical parameters (total carbohydrates, total glycogen, succinate dehydrogenase and lactate dehydrogenase).

The compound used for the study is a biopesticide—Anosom, manufactured by Agri Life India private limited, Hyderabad. The biopesticide was procured from the manufacturer. The biopesticide is a product extracted from the seeds of *Annona squamosa*. Its active ingredient is Annonin (squamosin). It is a liquid formulation containing Annonin in 10,000 ppm concentration.

Total carbohydrates and total glycogen were estimated by the method of Nicholas *et al.* (1956) using Anthrone reagent with some modifications. The SDH and LDH assays were estimated by modified method of Nachlas *et al.* (1960) with some modifications. For the estimation of total carbohydrates, 10 mg mL^{-1} (W V^{-1}) liver and 20 mg mL^{-1} (W V^{-1}) gill, kidney and muscle tissues were homogenized in 10% TCA. The homogenate was centrifuged at 3000 rpm for 15 min. One mL of clear supernatant was directly used for the estimation of total carbohydrates. Five ml of Anthrone reagent was added to each tube having 1 mL of clear supernatant, in an inclined position. All the tubes were capped and cooled down to room temperature. The colour developed was read against blank at 620 nm in a UV – visible spectrophotometer. The values obtained were expressed as mg of glucose/g wet wt. of tissue.

For the estimation of total glycogen, 10 mg mL^{-1} (W V^{-1}) liver and 20 mg mL^{-1} (W V^{-1}) gill, kidney and muscle tissues were homogenized in 10% TCA. The homogenate was centrifuged at 3000 rpm for 15 min. To 1 ml of supernatant, 5 ml of absolute ethanol was added. The tubes were capped and kept overnight in the refrigerator for complete precipitation. The tubes were then centrifuged at 3000 rpm for 15 min. The clear liquid was gently decanted from packed glycogen. The tubes were drained in an inverted position for 10 min. The glycogen obtained was dissolved in 1 ml of distilled water. Five ml of Anthrone reagent was added to each tube in an inclined position. All the tubes were capped and cooled down to room temperature. The colour developed was read against blank at 620 nm in a UV – visible spectrophotometer. The values obtained were expressed as mg of glycogen g^{-1} wet wt. of tissue.

For the estimation of SDH assay 200 mg mL^{-1} (W V^{-1}) gill and 100 mg mL^{-1} (W V^{-1}) liver, kidney and muscle tissues were taken and homogenized in 0.25 M ice-cold sucrose solution. The homogenate was centrifuged at 2000 rpm for 10 minutes. The clear supernatant was used for the enzyme assay. Two mL of incubation mixture consisted of 100 μ moles of phosphate buffer (pH 7.4), 2 μ moles of INT, 100 μ moles of sodium succinate (pH 7), 0.1 mL of distilled water and 0.5 mL of clear supernatant. The reaction mixture was incubated at 37° C for 30 min. The reaction was stopped by adding 4 mL of glacial acetic acid. The colour was extracted by adding 4 ml of toluene. All the tubes were shaken well, capped and kept in the refrigerator overnight. Formazon formed was measured at 490 nm against blank in a UV – visible spectrophotometer. The values obtained were expressed as μ moles of formazon formed mg^{-1} tissue hr^{-1} . For the estimation of LDH assay, 50 mg mL^{-1} (W V^{-1}) gill, liver, kidney and muscle tissues were taken and homogenized in 0.25M ice-cold sucrose solution. The homogenate was centrifuged at 2000 rpm for 10 minutes. The clear supernatant was used for the enzyme assay. Two ml of incubation mixture consisted of 100 μ

moles of phosphate buffer (pH 7.4), 2 μ moles of INT, 50 μ moles of sodium lactate (pH 7.4), 0.1 μ mole of NAD and 0.5 mL of clear supernatant. The reaction mixture was incubated at 37° C for 30 min. The reaction was stopped by adding 4 mL of glacial acetic acid. The colour was extracted by adding 4 ml of toluene. All the tubes were shaken well, capped and kept in the refrigerator overnight. Formazon formed was measured at 490 nm against blank in a UV –visible spectrophotometer. The values obtained were expressed as μ moles of formazon formed mg^{-1} tissue hr^{-1} .

All the results obtained were subjected to statistical analysis using IBM SPSS software version 21. The test used was one-way ANOVA. All the results are presented as mean \pm standard deviation at $p<0.05$ level of significance. The graphs were made using Microsoft Excel.

RESULTS

The fish during sub-lethal dose exposure i.e., 0.01 ppm to the biopesticide -Anosom showed the following results in gill, liver, kidney, and muscle when compared to the control group within 14 days. The results of the present study show that the carbohydrate metabolism in gill, liver, kidney, and muscle is disrupted on exposure to biopesticide – Anosom to some extent. The alterations were tissue specific and hence can be used as an important indicator of pesticide pollution. This type of study with fish provides useful information about the nature of the adverse effects of pesticides and biopesticides on aquatic biota especially fish, which constitute an important food source for human consumption. Hence, it is important to monitor the usage of every pesticide whether synthetic or biopesticide as both of them cause adverse effect on the health and well-being of the aquatic fauna.

Table 1: Total carbohydrate content (mg g^{-1}) in gill, liver, kidney and muscle tissues of *Cyprinus carpio* on exposure to Anosom

Tissue	Control	24 hrs	7 days	14 days
Gill	23.60 \pm 1.48	19.18 \pm 0.38*	15.39 \pm 0.74*	11.23 \pm 0.66*
Liver	164.20 \pm 1.92	151.02 \pm 0.91*	85.26 \pm 1.50*	68.20 \pm 1.11*
Kidney	27.92 \pm 0.56	23.13 \pm 1.56*	13.57 \pm 0.71*	10.60 \pm 0.48*
Muscle	71.90 \pm 0.65	56.65 \pm 0.82*	43.44 \pm 1.35*	30.63 \pm 0.69*

Values are expressed as mean \pm standard deviation. * $p<0.05$.

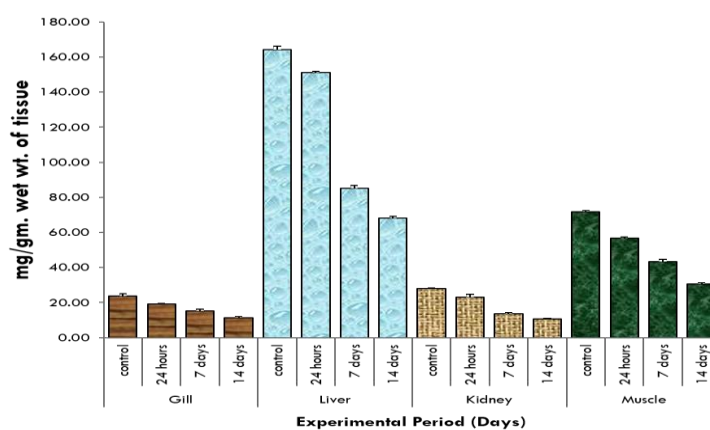


Figure 1. Total carbohydrate content in gill, liver, kidney and muscle of fish *Cyprinus carpio* during exposure to sub-lethal conc. of Anosom.

Table 2: Total glycogen content (mg g⁻¹) in gill, liver, kidney and muscle tissues of *Cyprinus carpio* on exposure to Anosom

Tissue	Control	24 hrs	7 days	14 days
Gill	13.86 ± 0.47	11.32±0.34*	8.97±0.73*	6.01±0.61*
Liver	56.10±0.61	50.59±0.43*	44.01±0.42*	33.44±0.42*
Kidney	14.78±0.50	11.45±0.60*	7.06±0.28*	4.56±0.46*
Muscle	30.66±0.49	27.63±0.41*	22.28±0.55*	16.80±0.57*

Values are expressed as mean ± standard deviation. **p*<0.05.

Figure 2. Total glycogen content in gill, liver, kidney and muscle of fish *Cyprinus carpio* during exposure to sub-lethal conc. of Anosom.

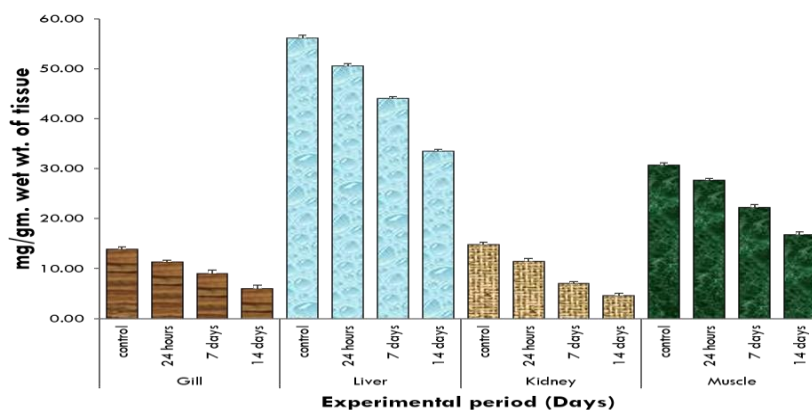


Table 3: Succinate Dehydrogenase (µmoles of formazan formed mg⁻¹ tissue hr⁻¹) in gill, liver, kidney and muscle tissues of *Cyprinus carpio* on exposure to Anosom

Tissue	Control	24 hrs	7 days	14 days
Gill	5.10±0.40	4.51±0.15*	3.51±0.23*	2.09±0.25*
Liver	8.76±0.72	6.51±0.92*	3.88±0.22*	2.63±0.64*
Kidney	12.16±1.45	9.67±0.46*	7.44±0.34*	5.54±0.46*
Muscle	10.41±0.36	8.99±0.13*	7.01±0.25*	5.04±0.16*

Values are expressed as mean ± standard deviation. **p*<0.05.

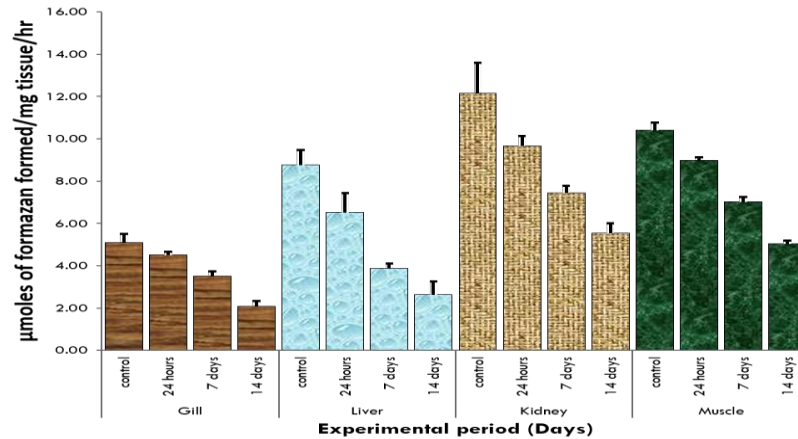


Figure: 3 S.D.H. conc. in gill, liver, kidney and muscle of fish *Cyprinus carpio* during exposure to sub-lethal conc. of Anosom.

Table 4: Lactate Dehydrogenase ($\mu\text{moles of formazan formed mg}^{-1} \text{ tissue hr}^{-1}$) in gill, liver, kidney and muscle tissues of *Cyprinus carpio* on exposure to Anosom

Tissue	Control	24 hrs	7 days	14 days
Gill	17.26±0.44	15.00±0.74*	12.35±0.69*	8.96±0.69*
Liver	35.14±0.73	30.73±0.60*	26.22±0.25*	20.24±0.80*
Kidney	31.40±0.64	25.62±0.33*	20.36±0.75*	15.12±0.66*
Muscle	18.53±0.57	14.72±0.47*	11.32±0.86*	8.83±0.48*

Values are expressed as mean \pm standard deviation. * $p < 0.05$.

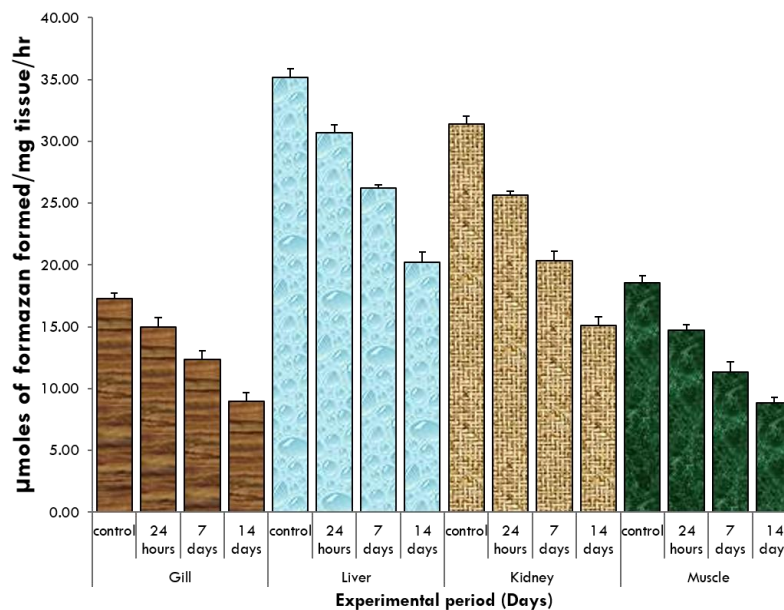


Figure: 4. L.D.H. conc. content in gill, liver, kidney and muscle of fish *Cyprinus carpio* during exposure to sub-lethal conc. of Anosom.

The total carbohydrate content was found to be highest in the liver, followed by muscle, kidney and gills, respectively. The concentration of total carbohydrates showed the significant decrease in all the four organs – gill, liver, kidney and muscle as the days of exposure increased. The total glycogen content was found to be highest in the liver followed by muscle, kidney and gill. The concentration of glycogen showed significant decrease in all four organs as the days of exposure increased. The total carbohydrate and glycogen value in all the four organs gill, liver, kidney and muscle was found to be highest in the control fish and least in fish of 14 days exposure. The SDH content was found to be highest in kidney, followed by muscle, liver and the gills. The concentration of SDH showed a significant decrease in all organs as the days of exposure increased. The LDH content was found to be highest in liver, followed by kidney, muscle and gill. The LDH concentration decreased significantly in all of the four organs as the days of exposure increased.

DISCUSSION

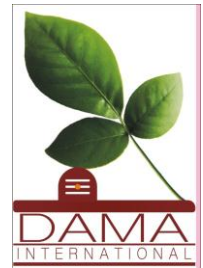
The results obtained from the present study show that the values of total carbohydrates, total glycogen, succinate dehydrogenase and lactate dehydrogenase in all the four organs – gill, liver, kidney and muscle decreased significantly after the completion of 24 hrs, 7 days and 14 days on exposure to sub-lethal concentration of Anosom. The results obtained in the present study are in correlation with the results obtained by many other researchers.

Gills are thin, fine respiratory structures which are in continuous contact with water and they carry out important functions like - gases exchange, ion regulation and excretion of metabolic wastes. As gills are in constant contact with the external environment, they are the first targets of waterborne pollutants (Perry and Lauvent, 1993). Liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants, it is involved in the synthesis of various proteins and is also a regulatory center of metabolism. Liver functions can be altered by changes caused during acute or chronic exposure to toxicants (Al- Attar, 2011). Kidney, the important organ of excretion and osmoregulation is indirectly affected by pollutants through blood circulation (Newman and McLean, 1974). Devi (1981) reported that the kidney is the site of degradation and detoxification of toxic substances. Muscle - rich in protein forms mechanical tissue intended for mobility and it does not participate in any metabolic activities. The impact of contaminants on the aquatic ecosystem can be assessed by measuring the biochemical parameters in fish that respond specifically to specific toxicant (Petrivsky *et al.*, 1997).

The study conducted in the present paper provides the evidence that like other kinds of synthetic pesticides, the biopesticides or botanical pesticides also affect carbohydrate metabolism in different tissues by altering the levels of metabolites and their associated enzymes. Carbohydrates are the first and immediate energy source to be utilized to a greater extent particularly in case of stress. Exposure to any kind of pollutant or toxicants results in stress which ultimately results in a reduction of total carbohydrates content in various tissues. Under stressful conditions, carbohydrate reserves are depleted in order to meet energy requirement by all tissues (Arasta *et al.*, 1996).

Under stress conditions, the energy demands are met by increased glycogenolysis which leads to decrease in tissue glycogen content (Wasserman *et al.*, 1970). The reduction in liver glycogen stores and carbohydrates could be due to reason that the liver synthesis of detoxifying enzymes requires high energy levels (Begum and Vijayaraghavan, 1995; Hori *et al.*, 2006). In pesticide-exposed fish the decrease in glycogen content clearly indicates its rapid utilization to meet the enhanced energy demands through glycolysis or hexose monophosphate pathway. Other reason for the decrease in glycogen content may be the inhibition of the enzyme glycogen synthetase. A decrease in tissue glycogen was observed in *Labeo rohita* on exposure to malathion and nuvan (Anuradha, 1993). Similar reduction in tissue glycogen was observed when *S. mossambicus* was exposed to DDT, malathion and mercury (Ramalingam, 1988). The findings of the present study are also in agreement with those of Bakhshwan *et al.* (2009).

SDH is one of the active regulatory enzymes of the TCA cycle. Decreased SDH activity clearly indicates the depression of TCA cycle i.e., depletion in the oxidative metabolism at the level of mitochondria. A similar decrease in the SDH activity was observed by Jacob *et al.* (2007) in freshwater fish exposed to cypermethrin. A similar trend of decrease in SDH activity was also reported in different organisms exposed to different chemicals (Sudharsan *et al.*, 2000; Al-Ghanim and Mahboob, 2012).



LDH is a potential marker enzyme for assessing the toxicity of pollutants and toxicants. Alterations in the LDH activity has been proven to be a very good marker and also serves as a diagnostic tool in toxicology studies for tissue damage in fish (Ramesh *et al.*, 1993), muscular damage (Balint *et al.*, 1997) and hypoxic conditions (Das *et al.*, 2004). LDH plays a very important role in carbohydrate metabolism by interconverting lactate and pyruvate (Lehninger *et al.*, 1993). LDH acts as a connecting enzyme between the glycolytic pathway and TCA cycle. Studies conducted by various researchers revealed that under toxicant exposure the LDH activity of various tissues gets altered (Tripathi *et al.*, 1990; Diamantino *et al.*, 2001; Mishra and Shukla, 2003; Rao, 2006; Agrahari and Gopal, 2009).

Alphamethrin exhibited alterations in biochemical parameters of *Channa punctatus* was observed by Tripathi and Singh (2013). According to Bhaskara and Vijaya (2016) Butachlor and Machete induced biochemical alterations in *C. punctatus*. In another study performed by Muddassir (2015), it was reported that Carbofuran and Malathion induced biochemical alterations in *C. punctatus*. Effect of Triclosan on total protein content in *C. punctatus* was studied by Ravi *et al.* (2015). Studies of Illiyas *et al.* (2016) concluded that Dimethoate affected physiology of *Catla catla* and *Labeo rohita*. Indoxacarb exhibiting alterations in biochemical parameters of *L. rohita* were recorded by Veeraiah *et al.* (2013). Effect of two pesticides on the biochemistry of *L. rohita* was investigated by Nagaraju and Venkata (2013).

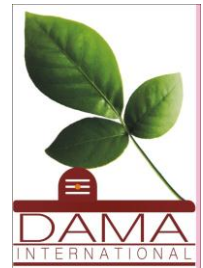
Cypermethrin induced biochemical changes in *Clarias batrachus* was reported by Prakash *et al.* (2014). Rather *et al.* (2015) thoroughly investigated the biochemical changes induced by carbaryl, carbosulfan and parathion in *C. batrachus*. Effect of fenthion on enzymes of *C. carpio* was studied by Leena (2014). Khalid (2014) studied the effect of cypermethrin on enzyme activities of *C. carpio*. Effect of phorate on the level of total proteins in *C. carpio* was investigated by Lakshmaiah (2014). Due to Cypermethrin, the enzymatic alterations in *C. carpio* were recorded by Neelima *et al.*, (2015). Tulasi and Jayantha Rao (2013) conducted a study to find the effect of chromium on protein metabolism of *C. carpio*. Similar work using trivalent chromium was done by Zeynab *et al.* (2013) in *C. carpio* and the biochemical profile was observed.

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