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To assess the impact of Cypermethrin (10 EC) on Natural fish food (zooplankton)

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Abstract

Agricultural pyrethroid insecticide, particularly cypermethrin, is frequently used in commercial carp aquaculture ponds to control aquatic bugs. These pyrethroids are extremely toxic to a broad spectrum of aquatic creatures. The aim of this study was to investigate the effect of pyrethroid insecticide cypermethrin (10 EC) on the survivability rate of Natural fish food (zooplankton). The eight different concentrations of cypermethrin (10 EC) were used 0.4, 14, 2.4, 3.4, 4.4, 5.4, 6.4, 7.4 μ l/l to investigate the cypermethrin toxicity. The stock solution was prepared by dissolving suitable amount of cypermethrin in one litre of diluent water. The test concentrations of dilute solution of cypermethrin were expressed in microliter per litre (μ l/l). At a concentration of 1.4 μ l/l of cypermethrin, the survivability rate was 82 % at the end of the 8th hour and 78% at the end of the 10th hour. At the concentrations of 6.4 μ l/l and 7.4 μ l/l, 60% and 50% survivability of zooplankton was reported by the end of 8th and 10th hour, respectively. The LC₅₀ values were recorded between 2.43 and 23.98 for different time durations.

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Keywords: Cypermethrin, Zooplankton, Toxicity, pyrethroid insecticide, survivability

1. Introduction

Pesticides are compounds, or combinations of compounds, intended to eliminate, repel, or prevent any organisms that pose a threat to people or their property. According to the Environmental Protection Agency (2001), pests include fungi, unwanted plants (weeds), mice, insects, and other microorganisms including bacteria and viruses. In aquaculture operations, pesticides are utilized for harvesting, pond preparation, and aquatic bug eradication (Kumar et al., 2023). The pyrethroids are a major class of highly active pesticides that are used worldwide to control pests in households, cereals, vegetables, cotton, tobacco, and other crops. They have high bio-efficacy and low toxicity compared to organochlorine and organophosphorous pesticides (Werner et al., 2002). They are also frequently used to manage the ectoparasites of domestic animals. For over a century, Pyrethrins have been produced from the blooms of Chrysanthemum cinerariaefolium, which have been utilized as insecticides (LaForge & Markwood, 1938). Pyrethyroids are more potent and environmentally stable, structural derivatives of pyrethrins (Casida, 1980; Elliott & Janes, 1978). According to Kaneko et al. (1978) and Kidd & James (1991), pyrethroids have been reported to break down quickly in the environment, while their half-life varies from one to sixteen weeks. Because they have a low mammalian toxicity and a high insecticidal toxicity, they are regarded as efficient insecticides (Elliott et al., 1974). Pyrethrins are extremely poisonous to fish and amphibian tadpoles and they mainly affect the skin mechanoreceptors and balance organs. These pyrethroids mediate their action by extending the open state of voltage-dependent sodium channels in nerve tissue (Narahashi, 2000; Soderlund et al., 2002; Vijverberg & Vanden, 1990). The principal physiological target of pyrethroid insecticides is the sodium channel, which is heavily reliant on stereochemical structure (Milam et al., 2000). Pyrethroid insecticides efficiently paralyze organisms by severely restricting neuro-transmission, by acting on the sodium channels to depolarize the presynaptic terminals (Salgado et al., 1983). It has also been demonstrated that pyrethroids prevent the synthesis of ATPase enzymes (Clark et al., 1989). Pyrethroids are easily absorbed by biological membranes and tissues because of their lipophilic nature. Exposed organisms may show signs of convulsions, tremors, and hyper excitation, followed by paralysis and drowsiness. Aquatic habitats may be impacted in a number of ways by pesticides in water, because of its lipophilic nature and tenacity, it bioaccumulates in aquatic creatures, such as fish and eventually finds its way into humans. Pesticide accessibility in fish will assist in the biomonitoring of pesticide-induced water pollution. Thus, fish can potentially be utilized as a bio-indicator to track pesticide pollution. It is possible for pesticides to be mobile i.e. they can move from source to source. These substances easily build in animal tissues and are persistent in food chains. Fish that consume contaminated food or drink water directly absorb these substances. These insecticides are the only effective way to control insects, however there are some drawbacks to the benefits of insecticides they provide. They are among the most hazardous compounds that could be introduced into the environment through surface runoff, air deposition, spraying, and direct application. Although insecticides are not species-specific and can impact non-target organisms just as easily as target organisms, it is not likely that a chemical that affects insects will have an equivalent effect on higher organisms (Kayhan et al., 2013). Acute toxicity, which is measured by the species-specific median lethal concentration (LC₅₀), is defined as a considerable decrease in the exposed organisms' survival rate in a comparatively short amount of time (Nikinmaa, 2014).

Cypermethrin, also known as {Cyano-(3-phenoxyphenyl) methyl}, is a cyanophenoxybenzyl pyrethroid that is classified as a restricted use pesticide (RUP) by the US Environmental Protection Agency due to its significant toxicity to fish. In 1974, cypermethrin was first produced (WHO, 1989). It acts fast, attacking the insect's central nervous system and causing its death after ingestion or touch (Sinha & Shrivastava, 2024). Despite being listed as a pesticide with restricted usage in India, cypermethrin is approved for application on a number of crops, including sugarcane, wheat, okra, brinjal, cotton, cabbage and sunflower (Patel *et al.*, 2018). In Andhra Pradesh, the cypermethrin pyrethroids make up over 70% of all the sprays used on cotton (Kranthi *et al.*, 2002). Cypermethrin was used in fish culture to prevent lice infestations (Jimenez *et al.*, 2013). Cypermethrin was in sixth place among India's most widely used native pesticides in 2009–2010. Pesticides find their way into water bodies through runoff from agricultural crops. Cypermethrin has half-life of more than 50 and 100 days, respectively, making it relatively stable to hydrolysis and photolysis in water (Jones, 1995). Because of its penetration and surface discharge into natural water bodies, cypermethrin has been shown to pose a significant risk to fishes (Raj *et al.*, 2014). Cypermethrin had been widely found in algae, fish, invertebrates (Vryzas *et al.*, 2011), sediments and water samples (Etchegoyen *et al.*, 2017), especially in regions with major agricultural practices.

Zooplankton makes up a significant amount of the living matter and plays an essential role in biogeochemical cycles in natural waters. Zooplankton is an important part of the food chain. In addition to being food for numerous fish, it also consumes bacterioplankton and phytoplankton (Kajak, 1998). Because of its significant role in the food chain and high sensitivity to toxins, zooplankton is commonly utilized in ecotoxicological tests. It is believed that zooplankton's reactions to toxicity tests provide insight into the overall effects on the ecosystem (Hanazato, 2001). In general, a wide variety of aquatic invertebrates, such as *Daphnia* species, Brachionus species, Gammarus species, and Ceriodaphnia species, are frequently employed in toxicity experiments (Fochtman et al., 2000). Due to their great sensitivity, ease of handling, and rapid reproduction, daphnids—particularly Daphnia magna—have long been employed in conventional toxicity testing (Hill, 1989). The OECD (1998) proposed a standardized chronic toxicity test employing *Daphnia*, which analyzed the reproduction (number of offspring generated) rate of tested animals. Reproduction analysis could be helpful in determining how chemicals affect population growth (Hanazato, 2001). Like other zooplankton, Brachionus sp. and Thamnocephalus platyurus are important food sources for a variety of fish species and aquatic invertebrates. Their easy culture, quick generation time, cosmopolitan distribution, and commercial availability of their dormant eggs have made them popular test organisms (Persoone et al., 1989). Because of their high sensitivity to synthetic pyrethroids, crustaceans typically exhibit acute toxicity levels of 1 µg/L or less. Zooplanktons (i.e., copepods, cladocerans, euphasids, and rotifers) are abundant and important crustaceans found in both freshwater and marine lentic environments. By recycling vital nutrients from biota and abiotic debris through ingestion and excretion, as well as by becoming prey for fish and other invertebrates, these zooplanktons play a crucial role in connecting trophic levels in aquatic food webs. The presence of synthetic pyrethroids in the aquatic environment may cause zooplankton to disappear or reduce in activity, which could have a significant impact on the composition and functionality of these ecosystems. Numerous studies have recently been published in the literature on the direct and indirect effects of pyrethroids on pelagic freshwater communities, including zooplankton, found in lake and pond mesocosms [Day *et al.*, 1987], as well as the acute and chronic toxicities of these chemicals to individual zooplankton species in the laboratory [Day & Kaushik, 1987].

2. Materials and Methods

2.1 Selection of optimum dosage of cypermethrin

The eight different concentrations of cypermethrin (10 EC) were used 0.4, 14, 2.4, 3.4, 4.4, 5.4, 6.4, 7.4 μ l/l to analyze cypermethrin toxicity. The stock solution was prepared by dissolving suitable amount of cypermethrin in one litre of diluent water. The experiment was repeated in triplicates in experimentation tanks. In tanks, the water was exchanged daily and a fresh cypermethrin concentration from stock solution was added to maintain the test concentration in tanks.

Table 1: Selection of optimum dosage of cypermethrin

Treatments	Treatments (Cypermethrin	No. of Tanks per			
	dosage)	treatment			
T ₀	Control	3			
T_1	0.4 (µl/l)	3			
T ₂	1.4 (µl/l)	3			
T ₃	2.4 (µl/l)	3			
T ₄	3.4 (µl/l)	3			
T 5	4.4 (µl/l)	3			
T ₆	5.4 (µl/l)	3			
T ₇	6.4 (µl/l)	3			
T ₈	2.4 (µl/l)	3			

2.2: Parameters Applied for Reporting the Data:

a) Median Lethal Concentration (LC_{50}):

The static bioassay tests were performed to calculate the median lethal concentration (LC₅₀), a concentration of cypermethrin at which 50 per cent of the test specimens survived for a specific time exposure. The LC₅₀ values were calculated at different concentrations of cypermethrin and time intervals (2, 4, 6, 8 and 10 hour) for cypermethrin in relation to each environment variables by Probit Analysis (Finney, 1971).

b) 95 per cent confidence limits:

The 95 per cent confidence limits, *i.e.* upper confidence limits (UCL) and lower confidence limits (LCL) and their ratios (R = UCL/LCL) for each LC_{50} were also calculated (Finney, 1971). The 95 per cent confidence limits indicate the accuracy of the estimate that would be expected from replicate of bioassay tests that were performed at the same time with the similar conditions.

c) Safe or harmless concentrations:

The presumable harmless or safe concentrations of cypermethrin for each variable in short –term toxicity for the test organisms were calculated by using the formula given by Hart *et al.*, 1945

 $C = (48 \text{ hour } LC_{50} \times 0.3) / S^{2}$ $24 \text{ hour } LC_{50}$ Where S^{2} = \times 1 $48 \text{ hour } LC_{50}$

C = is the harmless concentration and S indicates safe dischargeable concentration)

2.3: Toxicity tests:

- a) Short term toxicity tests: Short term toxicity tests were performed by following range finding tests which were further followed by short term definite tests.
- **b) Range finding tests:** The test chemical cypermethrin was applied at different concentrations and at time intervals of 2, 4, 6, 8 and 10 hours. The percentage survivabilities of zooplanktons were recorded at different concentrations of cypermethrin.
- c) Short- term definitive test: Short term definitive test was performed to determine the LC₅₀ or median lethal concentration of cypermethrin, in this static bioassay test, total eight different concentrations (0.4, 1.4, 2.4, 3.4, 4.4, 5.4, 6.4, 7.4 μ l/l) of cypermethrin were used to analyze the cypermethrin toxicity. Each cypermethrin concentration was applied in triplicate. The control tanks were kept away from bioassay tanks to avoid contamination. The survivability rates of zooplanktons were calculated at the end of 2,4,6,8 and 10

hours. The data recorded from the experiments was processed by probit analysis (Finney, 1971) for the calculation of LC₅₀ value through OPSTAT software and also by using graphical analysis. The slope function, 95% confidence limits (upper limit and lower limit) were calculated by using the response curve obtained for different exposure times (Reish *et al.*, 1987).

d)

2.4: Plankton count in cypermethrin treated water

For plankton's study, samples were collected in triplicate by filtration of 5 litre of water through plankton net of 50µm mesh size having a demarcated collection tube. This collection tube having 250 ml of capacity used for concentrated the sample. The plankton concentration so obtained was stored in 5 percent formalin.

2.5: Quantitative estimation of planktons

For quantitative analysis, plankton samples were counted using Sedgwick rafter counter cell. A total of 50 ml of sample was fixed with the help of 5 percent formalin. After that a concentrated sample of about 1.0 ml was transferred into the cavity of cell counter. Planktons were allowed to settled and 10 random selected fields were used for counting under the microscope. For each sample, the number of plankton counting was done by taking average of three readings by following the below formula.

Total Number of planktons (cells/litre) = $\frac{\text{Total no. of plankton counted} \times 1000 \times \text{V}}{\text{Volume of a filed (mm}^3) \times \text{F} \times \text{L}}$

Where:

V=Volume of final concentrate sample (ml)

F=Number of field counted

L=Original volume of water

2.6: Qualitative identification of plankton

The collected samples were observed under the high quality microscope (ECLIPSE Nikon TS 2) at 4x, 10x, 40x and 100x for identification of different kind of phytoplankton's and zooplanktons. One drop of fixed sample was placed on the slide and covered with the help of cover slip for qualitative analysis. Planktons were identified up to genus level using Edmondson (1974) and Needham & Needham (1962) manuals.

2.7: Statistical analysis

Data obtained during the experimental period was analysed by OPSTAT software using one way and two-way ANOVA. Results were expressed as mean \pm SE. Tukey's multiple range test was used to compare the mean differences. The regression equation was computed by probit analysis. The LC50 values of cypermethrin for test organisms were tested at 1% and 5% levels of significance (Snedecor and Cochram, 1980).

3. Results

The toxic effect of pyrethroid insecticide cypermethrin (10 EC) on the survivability rate of Zooplanktons is presented in the form of table and figures. For zooplankton analysis, water sample was collected by plankton net and fixed by adding 5% formalin then counting was done by using Sedgewick Rafter cell counter and visualized under different magnifications (4x, 10x, 40x and 100x) for their identification. Then zooplankton diversity was compared with control for assessing impact of cypermethrin.

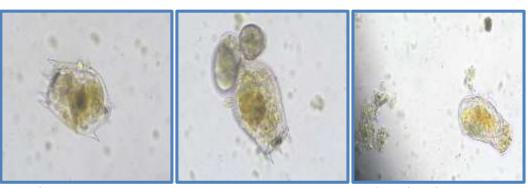
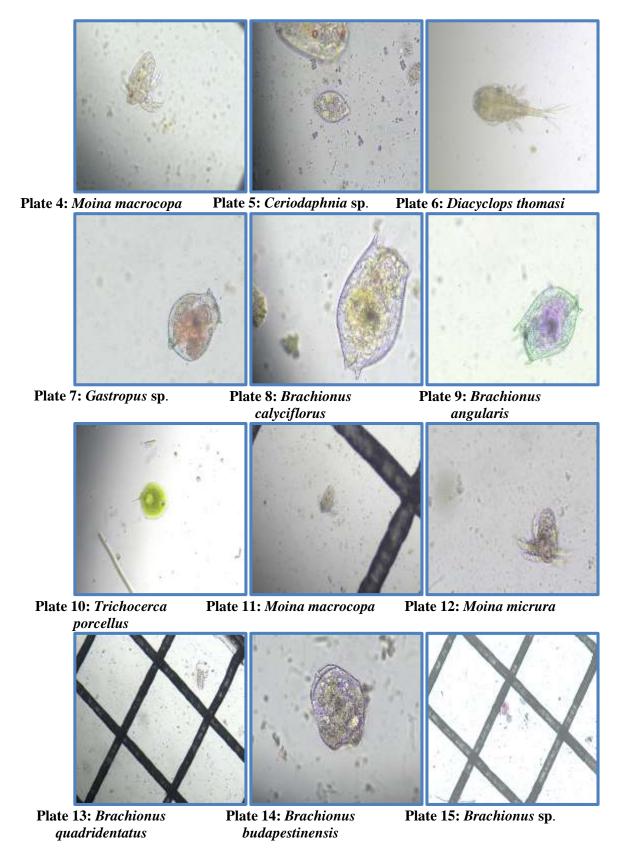
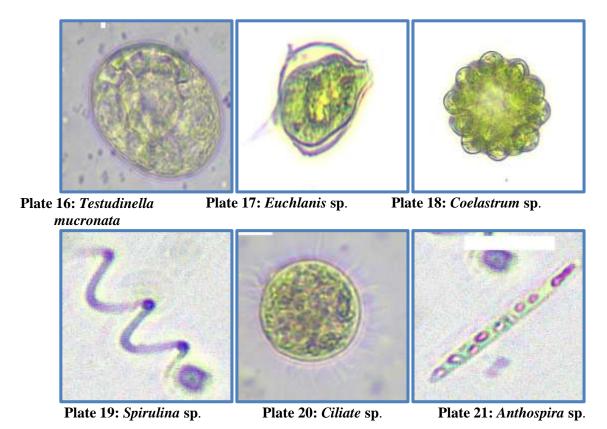


Plate 1: Brachionus sp.

Plate 2: Brachionus quadridentata

Plate 3: Polyarthra sp.





3.1: Plankton diversity

A wide variety of zooplanktons and phytoplanktons were reported in the cypermethrin treated water during the experimental period. Sixteen zooplanktons were identified among different treatments. The identified zooplanktons were: *Brachionus quadridentatus* (Plate 2), *Brachionus* sp. (Plate 1), *Brachionus calyciflorus* (Plate 8), *Polyarthra* sp. (Plate 3), *Diacyclops thomasi* (Plate 6), *Moina macrocopa* (Plate 11), *Ceriodaphnia* sp. (Plate 5), *Gastropus* sp. (Plate 7), *Brachionus angularis* (Plate 9), *Moina micrura* (Plate 12), *Trichocerca porcellus* (Plate 10), *Brachionus budapestinensis* (Plate 14), *Testudinella mucronata* (Plate 16), *Euchlanis* sp. (Plate 17). As shown in the pie chart (Figure 1), the identified zooplanktons belonged to 5 different classes involving Rotifera, Cladocera, Copepoda, Ostracoda and Diptera. Among all the classes, Rotifera (55%) was the most dominant class. Both Cladocera (28%) and Copepoda (15%) are the next most dominant classes. These were followed by Ostracoda (1%) and Diptera (1%). Additionally, some phytoplanktons like *Ciliate*, *Spirulina*, *Anthospira* and *Coelastrum* also identified in the cypermethrin treated water.

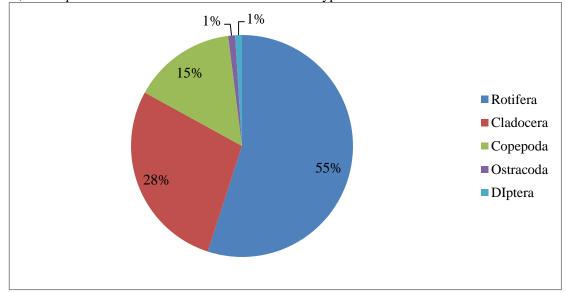


Fig. 1: Pie chart showing zooplankton diversity in cypermethrin treated water

3.2: Mean survivability (per cent) of zooplankton community at different concentrations of cypermethrin

The effect of different concentrations of cypermethrin on zooplankton community was reported. As presented in Table 2, 100% survivability was seen in zooplanktons within 4th hour in the initial dose of 0.4 μ l/l. The survivability rate decreased to 85 % by the end of 8th hr, and 82 % survivability occurred at the end of 10th hr. At a concentration of 1.4 μ l/l of cypermethrin, the survivability rate was 82 % at the end of the 8th hour and 78% at the end of the 10th hour. At 4.4 μ l/l concentration of cypermethrin, the survivability rate was 70 % by the end of 8th hour. At the concentrations of 6.4 μ l/l and 7.4 μ l/l, 60% and 50% survivability of zooplankton was reported by the end of 8th and 10th hour, respectively.

As presented in Table 3, The LC_{50} values were recorded between 2.43 and 23.98 for different time durations. The results showed that the 2^{nd} hr had the lowest LC_{50} values and 10^{th} hr had the highest LC_{50} values. The LC_{50} values for 4^{th} , 6^{th} and 8^{th} hr were 5.12, 9.34, and 14.45, respectively.

Table 2: Mean survivability (per cent) of zooplankton community at different concentrations of cypermethrin

cy per metri m											
Concentration of Survivability (%)											
Cypermethrin (µl/l)	2 nd hr	4 th hr	6 th hr	8 th hr	10 th hr	Mean survivability (%)					
Control	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100					
0.4	100.0±0.0	100.0±0.0	90.01±0.06	85.12±0.08	82.31±0.21	84					
1.4	100.0±0.0	90.11±0.31	90.32±0.22	83.24±0.72	79.25±0.82	82					
2.4	90.82±0.45	90.31±0.42	84.22±0.41	79.11±0.42	74.14±0.28	78					
3.4	82.26±0.42	80.34±0.26	78.12±0.24	72.22±0.56	70.54±0.12	76					
4.4	80.42±0.06	76.42±0.12	73.71±0.36	70.28±0.03	65.62±0.16	72					
5.4	74.12±0.35	70.13±0.92	70.26±0.36	64.14±0.32	60.21±0.32	68					
6.4	70.34±0.44	66.46±0.34	62.48±0.42	60.22±0.52	56.42±0.54	64					
7.4	68.43±0.05	62.13±0.07	60.52	52.11±0.52	50.28±0.05	48					
Mean	88.79	82.98	75.33	63.68	58.84						

Table 3: LC₅₀ of cypermethrin at different time periods for zooplankton community

S.	Name of Parameters		Time interval (hrs.)					
No.		2 nd hr	4 th hr	6 th hr	8 th hr	10 th hr		
1.	LC ₅₀	2.43	5.12	9.34	14.45	23.98		
2.		4.61	4.356	3.62	2.99	2.602		
	95% lower confidence limits for log cypermethrin concentrations	0.663	0.631	0.558	0.475	0.416		
4.	95% upper confidence limits for cypermethrin concentrations	4.88	4.673	4.106	3.53	3.365		
5.	95% upper confidence limits for log cypermethrin concentrations	0.68	0.669	0.613	0.547	0.526		
6.	Regression coefficient	-0.0063	-0.0037	-0.0027	-0.0013	-0.0021		
7.	Standard error	0.06	0.08	0.11	0.19	0.28		

4. Discussion

In the present research, the effect of different concentrations of cypermethrin on zooplankton community was observed. 100% survivability was seen in zooplanktons within 4th hour in the initial dose of 0.4 μ l/l. The survivability rate decreased to 85 % by the end of 8th hr, and 82 % survivability occurred at the end of 10th hr. At a concentration of 1.4 μ l/l of cypermethrin, the survivability rate was 82 % at the end of the 8th hour and 78% at the end of the 10th hour. At 4.4 μ l/l concentration of cypermethrin, the survivability rate was 70 % by the end of 8th hour. At the concentrations of 6.4 μ l/l and 7.4 μ l/l, 60% and 50% survivability of zooplankton was reported by the end of 8th and 10th hour, respectively. The present study aligns with the finding of Christensen *et al.* (2005) reported that the swimming ability of Daphnia *magnia* got disrupted at cypermethrin concentrations greater than 0.1 μ g/l after 6, 24 and 48 h of exposure. In a similar finding by Medina *et al.* (2004) stated the effect of cypermethrin concentrations on zooplankton community. The zooplankton population was observed both pre- and post-cypermethrin treatment. Cypermethrin considerably reduced (P < 0.05) the overall abundance of zooplankton. Cypermethrin reduced the abundance of all major

taxonomic groups (>5 individuals L⁻¹) with the exception of bivalve larvae. Further investigation showed that there were no considerable (P < 0.05) variations in the population of copepods, cladocerans, and rotifers between enclosures prior to treatment but there were considerable changes in population (P < 0.05) of these organisms after cypermethrin treatment. Lutnicka *et al.* (2014) demonstrated the effect of two pyrethroids, cypermethrin and deltamethrin in very low concentrations (0.02 μ g l⁻¹) on zooplanktons. By comparing the low amounts of each pyrethroid to the control group in the *Daphnia magna* reproduction test (21 days), it exhibited no significant differences. Cypermethrin at concentration 0.13 μ g l⁻¹ affected the zooplankton community after 11-day exposure duration. Gottardi *et al.* (2017) reported that the population growth rate of *Daphnia* sp. did not have significant effect at cypermethrin concentrations of 0.002 – 0.02 μ g/l after 21 days of exposure. According to Martins *et al.* (2007), *Daphnia magna* was immobilized by deltamethrin at values between 0.05 and 1.01 μ g l⁻¹ (24-hour EC50) and 0.27 to 4.65 μ g l⁻¹ (48-hour EC50). Cypermethrin at a concentration of 0.002-0.2 μ g l⁻¹ had no effect on population growth rate of *Daphnia magna* after 21 days' exposure duration.

The LC₅₀ values of cypermethrin were recorded between 2.43 and 23.98 for different time durations. The results showed that the 2nd hr had the lowest LC₅₀ values and 10th hr had the highest LC₅₀ values. The LC₅₀ values for 4th, 6th and 8th hr were 5.12, 9.34, and 14.45, respectively. The similar study carried out by Mugni et al. (2013) reported that the 24h LC₅₀ values of cypermethrin for Brachionus calyciflorus was found 80. Planktons play an important role in fish culture by utilizing the accumulated nitrogenous products in the culture water to synthesize sugars and proteins, as well as provide oxygen in the presence of light (Khanjani et al. 2023). In the present study, the control had significantly higher plankton count as compared to the cypermethrin treated groups. A wide variety of zooplanktons and phytoplanktons were reported in the cypermethrin treated water during the experimental period. Sixteen zooplanktons were identified among different treatments. The identified zooplanktons were: Brachionus quadridentatus, Brachionus sp., Brachionus calyciflorus, Polyarthra sp., Diacyclops thomasi, Moina macrocopa, Ceriodaphnia sp., Gastropus sp., Brachionus angularis, Moina micrura, Trichocerca porcellus, Brachionus quadridentatus, Brachionus budapestinensis, Euchlanis sp., Testudinella mucronata. As shown in the pie chart (Figure 1), the identified zooplanktons belonged to 5 different classes involving Rotifera, Cladocera, Copepoda, Ostracoda and Diptera. Among all the classes, Rotifera (55%) was the most dominant class. Both Cladocera (28%) and Copepoda (15%) are the next most dominant classes. These were followed by Ostracoda (1%) and Diptera (1%). This study correlates with the findings of Day (1989) demonstrated the effect of four pyrethroids on cypermethrin, permethrin, deltamethrin and fenvalerate on zooplanktons. For cladocerans and copepods, the range of acute toxicities was reported to be 0.12 to 5.0 µg/L. Daphnid reproduction and food filtering rates were decreased at lower pyrethroid concentrations (50.01 µg/L). Cladoceran populations were decreased at these cypermethrin concentrations (>1 µg/L). Cladoceran filtration rates were reduced at concentrations of 0.05 µg/L or higher. Copepods, rotifers, and ostracods were decreased in response to higher concentrations levels (>10 μg/L) of cypermethrin. Zooplankton abundance was unaffected by pyrethroid concentration of 0.01 μg/L.

5. Conclusion

The bioassay studies were conducted using seven concentrations of cypermethrin (0.4, 1.4, 2.4, 3.4, 4.4, 5.4, 6.4, 7.4 μ l/l). The LC₅₀ values of cypermethrin for test organisms was calculated to analyse the cypermethrin toxicity. It was further reported that the low concentrations of cypermethrin did not affect the survival rate of zooplanktons. When comparing the findings of our investigation with those of earlier studies, it is challenging to draw firm conclusions on the harmful effects of low and extremely low concentrations of pyrethroid cypermethrin on aquatic life. In our study, a very low concentration (0.4 μ l/l) of cypermethrin did not cause any detectable and statistically significant toxic effects on survivability rate of zooplanktons at initial exposure. It is likely that different experimental settings contributed to the variations in the data that the researchers collected. Pyrethroid sensitivity depends on the species being tested, the size and age of organism, the type of pyrethroid being tested, the exposure duration, etc.

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