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Study on the Effects of Organophosphate Insecticide Triazophos, Biopesticide Spinosad and a Pyrethroid Insecticide Cypermethrin on Oxidative Stress Biomarkers of *Branchiura sowerbyi* (Beddard, 1892)

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ABSTRACT

This study aims to evaluate the toxic effects of organophosphate insecticide triazophos, biopesticide spinosad, and a pyrethroid insecticide cypermethrin on benthic Oligochaete worm, *Branchiura sowerbyi* during 96 h acute exposure. *B. sowerbyi* were exposed to two different sub-lethal concentrations (10% and 50% of 96h LC_{50}) of triazophos, spinosad, and cypermethrin for 96 h in laboratory conditions. Catalase (CAT) activity of the control and treated worms were evaluated after 24 and 96 h of exposure. Integrated biomarker response (IBR) was applied for comparison between these three toxicants. For all sub-lethal doses i.e. 2.25 $mg.L^{-1}$ and 0.5 $mg.L^{-1}$ of test chemical triazophos, 3.07 $mg.L^{-1}$ and 0.6 $mg.L^{-1}$ of test chemical spinosad, and 0.38 $mg.L^{-1}$ and 0.08 $mg.L^{-1}$ of test chemical cypermethrin, catalase (CAT) activity raised significantly ($p<0.05$) in the treated worms in compare to the control worms. This study shows that toxicants including Triazophos, spinosad, and cypermethrin cause a large increase in catalase (CAT) activity in *Branchiura sowerbyi*, which is likely due to the toxicant's increased ROS creation neutralizing the negative effects. IBR analysis aids in the differentiation of these three compounds' harmful effects. As per IBR analysis, the rank of the toxicants is Cypermethrin > Triazophos > Spinosad.

INTRODUCTION

The chemicals which are designed specially to destroy unwanted species are termed pesticides. These pesticides are drained into water bodies and severely affect the life of aquatic species, in addition to destroying the target organisms (Pereira et al. 1996). According to an estimate, more than 200 toxic chemical pesticides are used in agriculture in various parts of the world (Mishra & Bohidar 2005). Pesticide use is increasing at an alarming rate across the world, posing a significant threat to the ecosystem. Pesticide residues in food are a global problem (Uno et al. 2001). Around 25 percent of the pesticides in the world are used by developing countries (WHO 2003). Except for Japan, India is the largest producer of pesticides in South Asia and Africa and ranks twelfth in the world for the use of pesticides (Abhilash & Singh 2009).

Organophosphate pesticides have been widely used in recent years due to rapid biodegradation and limited retention time in the field. Triazophos or O,O-diethyl, 0.1 phenyl, 1H, 1,2,4, triazol 3yl-phosphorothio is a broad-spectrum systemic insecticide and acaricide also. Triazophos is the most carelessly used insecticide of these pesticides, affecting non-target species such as finfishes, shellfishes, etc. (Reddy et al. 1983). It undergoes biomagnifications across the food chain due to the persistent presence of Triazophos in the ecosystem, creating significant concern for human health. Experiments on the adverse effect of Triazophos on freshwater crabs have shown major changes in the enzymes action pathways of various metabolic reactions (Geethanjali 1985). spinosad is a new type of natural-origin insecticide or biopesticide. spinosad is produced by one or more chemical mutants of *Saccharopolyspora spinosa*, the naturally occurring actinomycetes soil bacterium (Mertz & Yao 1990) spinosad has been used to control insects from the Lepidoptera, Diptera, and Thysanoptera orders, as well as certain Coleoptera and Orthoptera species (Thompson et al. 2000). It's a stomach toxin with some contact action, and it has some control over little beetle larvae (Thompson et al. 2000). The nicotinic receptors activate the acetylcholine nerve system, which kills insects. Some effects on GABA and other nervous systems

may occur (Salgado 1997, 1998). Synthetic pyrethroids were developed for public health and agricultural purposes in the 1970s. But the use has grown over the last two decades. They are non-systemic insecticides. Cypermethrin is a type-II pyrethroid that acts by blocking the Na channels and by affecting the activity of GABA nerve filament receptors (Dobsikova et al. 2006). In addition to pests, the ecology of non-target species often appears to be affected. With 96 h LC_{50}s ranging from 0.01–5.0 $\mu g.L^{-1}$, cypermethrin is a strong toxic agent for fish and many aquatic non-chordates (Sarkar et al. 2005, Carriquiriborde et al. 2007).

These pesticides enter the water bodies through agricultural runoff water and impact the non-target organisms (Usman et al. 2020). Because Tubificid worms are sedentary and extensively spread, they can easily overwhelm the macrobiotic population in freshwater habitats, making them a valuable water pollution biomonitor. Furthermore, because they live in the soil, burrowing and ingesting large amounts of sediment, they are subject to pollution from both interstitial water and particle interaction. They frequently play a prominent part in bioturbation and inorganic matter decomposition (Lagauze`re et al. 2009). There is no evidence of the effects of various pesticides on the enzymatic activities of this worm. While *Branchiura sowerbyi* has been proposed for ecotoxicological studies as a test animal, there is no data available on the effects of various pesticides on the enzyme activities of this worm. However, very little evidence is available on the impact of various toxic substances on other Tubificid worms (Gillis et al. 2002, 2004, Maestre et al. 2009, Lobo et al. 2016).

Exposure to xenobiotics results in reactive oxygen species or ROS formation. Oxidative stress is caused by an imbalance in the ratio of pro-oxidants and antioxidants. Toxic substances such as insecticides, heavy metals as well as herbicides are toxic since the development of ROS causes oxidative stress in aquatic organisms (Usman et al. 2020). Oxidative stress occurs mainly through the development of reactive oxygen species (ROS) and can damage lipids, proteins, and DNA, leading to enzyme loss of function and structural integrity, and can cause inflammatory processes (Ozyurt et al. 2004). In most cases, an exceptional concentration of ROS, which might result in significant degeneration of the cell structure, is regarded as an effective oxidative damage signal (Barzilai & Yamamoto 2004). Oxidative damage is caused by three factors: (a) a rise in the generation of oxidants, (b) a decrease in the availability of antioxidants, and (c) a failure to mitigate oxidative harm (Das et al. 2010). Superoxide (O^{-}_{2}), one of the parent forms of reactive oxygen species, is a very reactive molecule, but can be converted by superoxide dismutase (SOD) to H_2O_2 and then by enzymes such as catalase (CAT) to oxygen and water (Klotz & Steinbrenner 2017). The analysis of changes in the activity of antioxidant enzymes such as CAT could therefore be an efficient way of marking oxidative stress, and changes in their activity and other biomarkers may be a potential tool in the aquatic toxicological study (Kumari et al. 2014, Kiliç & Kiliç 2017).

In evaluating the health of aquatic organisms, several researchers have proposed the use of oxidative biomarkers and more in-depth studies are required to evaluate an exact cause-effect relationship (Nussey et al. 2000, Vutukuru 2003). But very limited data is available concerning the sublethal toxicity of different pollutants on *Branchiura sowerbyi* (Bhattacharya et al. 2021b). There is no previous report on the toxicity of organophosphate Triazophos, biopesticide spinosad, and synthetic pyrethroid insecticide cypermethrin in *B. sowerbyi*. Hence, a laboratory study of the tubificidal worm *B. sowerbyi* as a function of the organophosphate insecticide Triazophos, biopesticide spinosad, and synthetic pyrethroid insecticide cypermethrin was therefore carried out to study the efficacy of this method. This research intends to study the oxidative stress induced by organophosphate pesticide Triazophos, Biopesticide spinosad, and synthetic pyrethroid insecticide cypermethrin on *Branchiura sowerbyi*, the most common benthic worm of Oligochaeta as well as an important fish food organism. Finally, integrated biomarker response (IBR) analysis was conducted for a better understanding of the toxic effects of these selected pesticides on *B. sowerbyi*. The knowledge gained will further assist in formulating strategies for treating contaminated water bodies with agriculture runoff and keeping aquatic bodies safe for aquatic life to exist.

MATERIALS AND METHODS

Branchiura sowerbyi (Phylum: Annelida, Class: Clitellata, Order: Oligochaeta and Family: Naididae) were obtained from a local unpolluted source, North 24 Parganas, West Bengal, India, and acclimatized in stock aquariums for 24 h in continuously aerated unchlorinated water (temperature 26.8 ± 0.5°C, pH 7.6 ± 0.2, free CO_2 12.5 ± 0.5 $mg.L^{-1}$, dissolved oxygen 6.4 ± 0.7 $mg.L^{-1}$, total alkalinity 185 ± 5.6 $mg.L^{-1}$ as $CaCO_3$, hardness 125 ± 5.2 $mg.L^{-1}$ as $CaCO_3$). The commercial formulation of an organophosphate pesticide Triazophos was an emulsified concentrate (EC) containing 40% a. i. (40% EC), biopesticide spinosad (45% SC), and synthetic pyrethroid insecticide cypermethrin (10% EC), which were procured from the market. Test chemicals were dissolved in pure distilled water to make a stock solution of 1 $g.100\ mL^{-1}$ (1% w/v) each. The 96 h median lethal concentration (LC_{50}) of Triazophos, spinosad and cypermethrin to *Branchiura sowerbyi* was previously recorded using a static

renewal acute toxicity test when the worms were applied to multiple nominal triazophos concentrations (0.0, 4.0, 4.32, 4.80, 5.60, 5.92, 6.40, 7.20, 8.00, 8.80, 9.60, 10.40 and 11.20 $mg.L^{-1}$), nominal spinosad concentrations (0.0, 3.2, 3.6, 7.2, 10.8, 14.4, 18.0, 21.6, 27.0, 30.6, 36.0 and 36.2 $mg.L^{-1}$) and nominal cypermethrin concentrations (0.0, 0.2, 0.8, 1.4, 1.8, 2.4, 2.8, 3.2, 3.6, 3.8 and 4.0 $mg.L^{-1}$) over a 96 h duration. All the required doses of test chemicals were made by adding the required amounts of triazophos, spinosad, and cypermethrin with unchlorinated tap water. The 96 h LC_{50} values of triazophos, spinosad, and cypermethrin for *Branchiura sowerbyi* were experimented and reported to be 5.04 (4.29-5.60), 6.14 (3.98-8.22) and 0.75 (0.39-1.09) $mg.L^{-1}$ respectively by the method of Finney's probit analysis (Sarkar et al. 2016, Sarkar & Saha 2017, 2018). To determine the oxidative stress at sub-lethal level, 2 g of *B. sowerbyi* (mean length of 11.1 ± 0.4 mm) were transferred from the stock into glass beakers (made by Borosil) each containing 1 L of unchlorinated tap water. The physicochemical parameters of water were monitored daily in the experimental bioassay throughout the exposure period (temperature 28.8 ± 0.5 °C, pH 7.5 ± 0.5, free CO_2 12.5 ± 0.5 $mg.L^{-1}$, dissolved oxygen 5.8 ± 0.5 $mg.L^{-1}$, total alkalinity 171 ± 7.4 $mg.L^{-1}$ as $CaCO_3$, hardness 125 ± 5.6 $mg.L^{-1}$ as $CaCO_3$). *Branchiura sowerbyi* was then exposed in separate beakers containing triazophos at concentrations of 50% of its 96 h LC_{50} value (2.25 $mg.L^{-1}$) and 10% of its 96 h LC_{50} value (0.5 $mg.L^{-1}$), 50% of its 96 h LC_{50} value (3.07 $mg.L^{-1}$) and 10% of its 96 h LC_{50} value (0.6 $mg.L^{-1}$) of test chemical spinosad and 50% of its 96 h LC_{50} value (0.38 $mg.L^{-1}$) and 10% of its 96 h LC_{50} value (0.08 $mg.L^{-1}$) of test chemical cypermethrin. Along with the treatments, a separate control group of non-exposed test organisms was also maintained. The duration of this experiment was 96 h. On the first day of the experiment, initial doses were treated. 10% of test water was replaced every 24 h by stock water and 10% of the initial concentration of three test chemicals was added immediately to test the water to make a fixed concentration. An aerator was used to provide continuous aeration during the experiment. Three replicates were used in the experiment. The same method was followed by previous authors (Bhattacharya et al. 2021b, Chatterjee et al. 2021). From each replicate, 1 g of test organism was taken at every exposure time i.e. 24, 48, 72, and 96 h followed by homogenization in 0.1 M phosphate buffer at pH 7.6. The homogenate was then centrifuged through HERMLE Labortechnik for 10 min @ 10000 rpm. The resultant product or supernatant was preserved at −20 °C till further examination. Catalase (CAT) activity was measured using a standard protocol (Beers & Sizer 1952) after the melting of hydrogen peroxide. CAT units are expressed as units of activity per milligram of protein (U/mg protein). The parameters were measured at room temperature (28°C) with a UV visible spectrophotometer (Cecil Aquarius 7400 CE). After subsequent verification of normality using the Shapiro Wilk Test, comparisons between control and exposed worms were conducted through two-way ANOVA followed by Tukey Test. Integrated biomarker response (IBR) analysis was carried out as per Beliaeff and Burgeot (2002). The result of the IBR analysis was presented in a star plot. Results are summed up as mean ± standard deviation (SD). It was agreed that the degree of statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

In the case of triazophos, CAT activity increased significantly in a concentration and duration-dependent manner both initially and gradually compared to control (Fig. 1; $p<0.05$). At a concentration of 2.25 $mg.L^{-1}$ during the 96 h exposure period, the highest induction in CAT activity was observed. Significant differences ($p<0.05$) between groups treated with Triazophos (0.5 and 2.25 $mg.L^{-1}$) were observed within each exposure period (24 and 96 h).

In the case of spinosad, CAT activity increased significantly in a concentration and duration-dependent manner both immediately and gradually in comparison to control (Fig. 2; $p<0.05$). At a concentration of 3.07 $mg.L^{-1}$ during the 96h exposure period, the highest induction in CAT activity was observed. Significant differences ($p<0.05$) between groups treated with spinosad (0.6 and 3.07 $mg.L^{-1}$) were observed within each exposure period (24 and 96h).

In the case of cypermethrin, CAT activity increased significantly in a concentration and duration-dependent way both immediately and gradually in comparison to control (Fig. 3; $p<0.05$). At a concentration of 0.38 $mg.L^{-1}$ during the 96 h exposure period, the highest induction in CAT activity was observed. Significant differences ($p<0.05$) between groups treated with cypermethrin (0.08 and 0.38 $mg.L^{-1}$) were observed within each exposure period (24 and 96 h).

In the case of cypermethrin, triazophos, and spinosad, T2-96 h is the most affected group and as per the IBR index, the rank of the most affected groups was: T2-96 h > T1-96 h > T2-24 h > T1-24 h (Fig. 4) whereas all control groups (C-24 h and C-96 h) remains unaffected.

As per IBR analysis based on the toxic effects of the selected toxicants on catalase activity of *Branchiura sowerbyi* after 24 and 96 h of exposure, the rank of these three toxicants could be ordered as cypermethrin > triazophos > spinosad (Fig. 5).

A complex equilibrium is maintained between the generation of ROS and cellular antioxidative defense enzymes under normal circumstances (Kamel et al. 2012). Pesticides

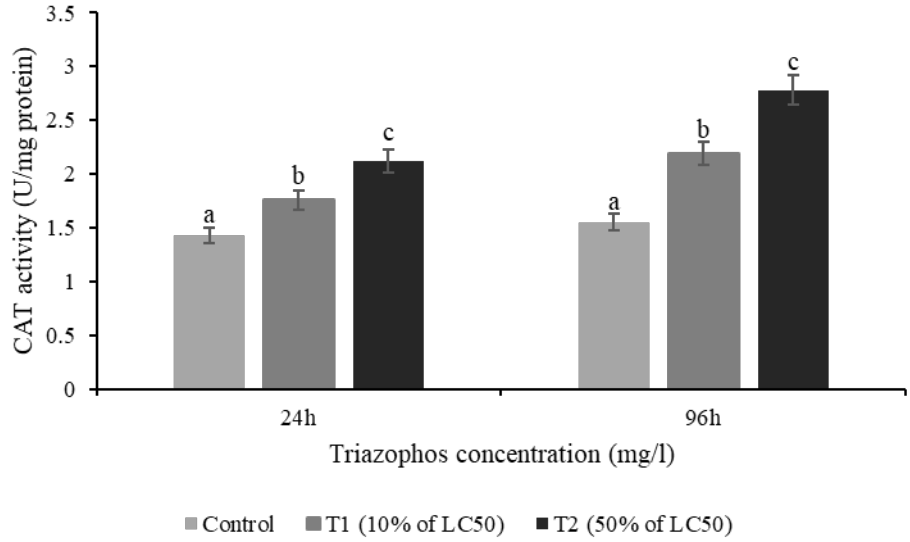


Fig. 1: Effects of different Triazophos sublethal concentrations on catalase (CAT) levels in *Branchiura sowerbyi* during different periods of exposure (24, 48, 72, and 96 h). A significant difference within the same exposure period is indicated by different letters (a-c) ($p < 0.05$). T1 shows the concentration of triazophos at 10% of its 96 h LC_{50} value (0.5 $mg.L^{-1}$); T2 shows the concentration of triazophos at 50% of its 96 h LC_{50} value (2.25 $mg.L^{-1}$). Different letters (a-c) indicate a significant difference ($p < 0.05$) within the same exposure period.

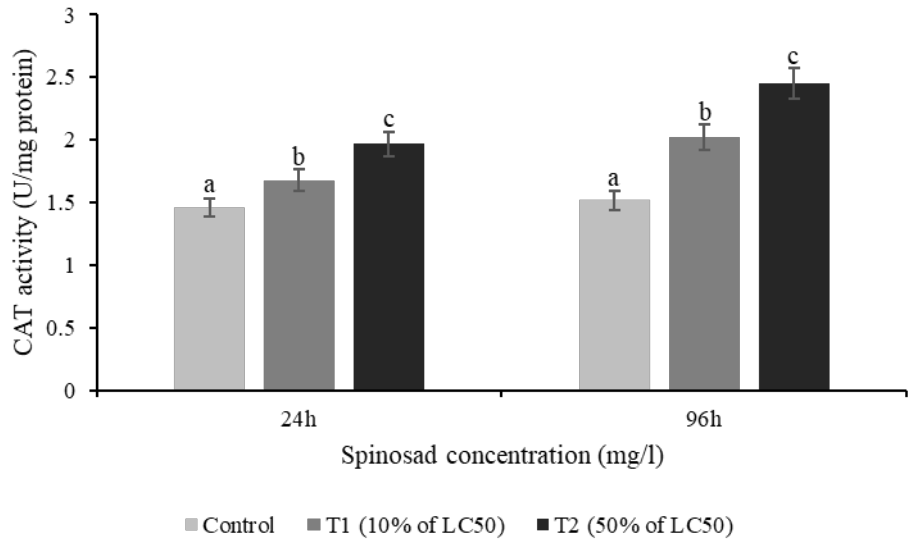


Fig. 2: Effects of different spinosad sublethal concentrations on catalase (CAT) levels in *Branchiura sowerbyi* during different periods of exposure (24, 48, 72, and 96 h). A significant difference within the same exposure period is indicated by different letters (a-c) ($p < 0.05$). T1 shows the concentration of spinosad at 10% of its 96 h LC_{50} value (0.6 $mg.L^{-1}$); T2 shows the concentration of spinosad at 50% of its 96 h LC_{50} value (3.07 $mg.L^{-1}$). Different letters (a-c) indicate a significant difference ($p < 0.05$) within the same exposure period.

can cause oxidative damage in species by disrupting ideal redox homeostasis, which can lead to physiological, biochemical, and morphological alterations (Chatterjee et al. 2021). Among different antioxidant enzymes, CAT (catalase) is an essential enzyme in the antioxidant system of organisms primarily involved in the detoxification of ROS and H_2O_2 degradation of molecular oxygen and water (Pandey et al. 2001, Usman et al. 2020). An organophosphate insecticide triazophos, biopesticide spinosad and a pyrethroid insecticide cypermethrin induced a substantial increase in catalase activity in the current experiment, which is possibly due to the neutralization of the harmful effect of increased ROS

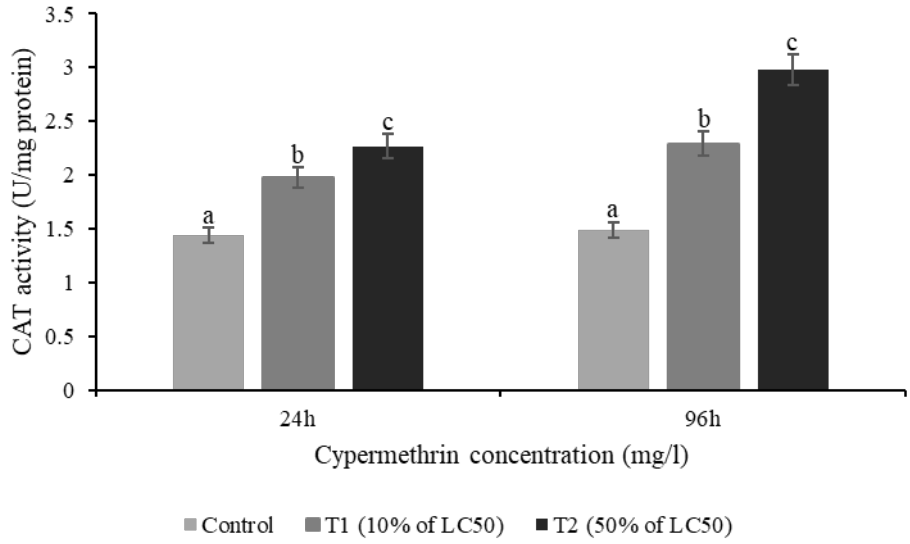


Fig. 3: Effects of different cypermethrin sublethal concentrations on catalase (CAT) levels in *Branchiura sowerbyi* during different periods of exposure (24, 48, 72, and 96 h). A significant difference within the same exposure period is indicated by different letters (a-c) ($p < 0.05$). T1 shows the concentration of cypermethrin at 10% of its 96 h LC_{50} value (0.08 $mg.L^{-1}$); T2 shows the concentration of cypermethrin at 50% of its 96 h LC_{50} value (0.38 $mg.L^{-1}$). Different letters (a-c) indicate a significant difference ($p < 0.05$) within the same exposure period.

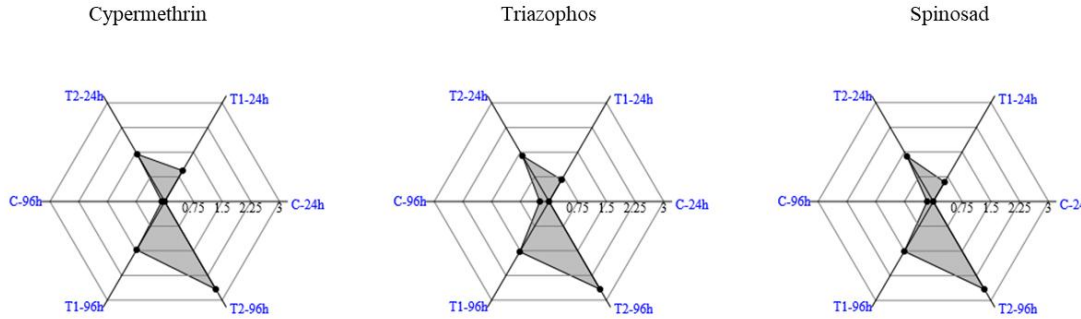


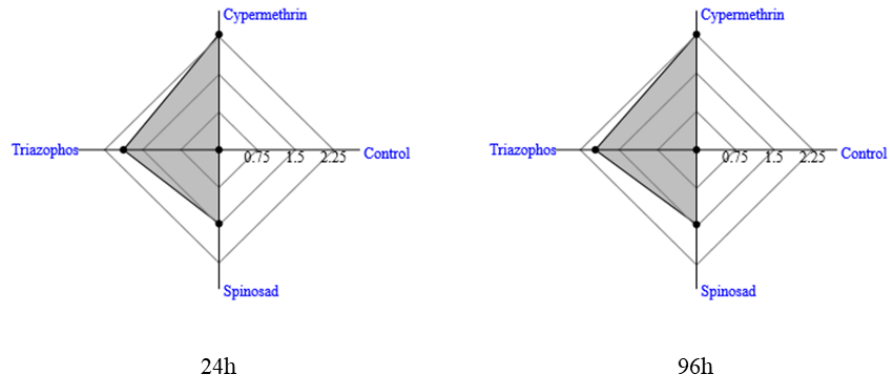
Fig. 4: Integrated Biomarker Response (IBR) of Catalase measured in *Branchiura sowerbyi* after sublethal exposure to cypermethrin, triazophos, and spinosad. C indicates control (0 $mg.L^{-1}$), T1 indicates the concentration of the toxicant at 10% of its 96 h LC_{50} value; T2 indicates the concentration of the toxicant at 50% of its 96 h LC_{50} value.

generation induced by the toxicant (Kumari et al. 2014). A comparable form of an increase in CAT activity in *Tubifex tubifex* after exposure to different pesticides was also observed by previous researchers (Mosleh et al. 2014, Di et al. 2016, Bhattacharya et al. 2021a, Chatterjee et al. 2021,).

Integrated biomarker response (IBR) analysis is a very useful method for measuring contaminants in the water ecosystems and is very useful for evaluating environmental stress (Li et al. 2011, Chang et al. 2020). A higher IBR value than the control indicates that the environment is more stressful for the species, whereas a lower value indicates optimum conditions (Bhattacharya et al. 2020). IBR analysis revealed that larger concentrations of these selected toxicants generate more stressful conditions for the organism than lower concentrations and that the stress increases as the exposure period increases.

CONCLUSION

Results of this experiment showed that *Branchiura sowerbyi* exhibited alterations in oxidative stress parameters with the integration of an organophosphate insecticide triazophos, biopesticide spinosad, and a pyrethroid insecticide cypermethrin. As a result, current studies on the toxicity of organophosphate insecticide triazophos, biopesticide spinosad, and pyrethroid insecticide cypermethrin to *Branchiura sowerbyi* indicate that oxidative stress biomarkers are an important method for assessing the intricately designed health status of aquatic species, including benthic macro-invertebrate



24h 96h

Fig. 5: Integrated Biomarker Response (IBR) for catalase activity in *Branchiura sowerbyi* after 24 and 96 h of sublethal exposure to cypermethrin, triazophos, and spinosad.

animals under stress. The pyrethroid pesticide cypermethrin caused more stressful conditions followed by the organophosphate, triazophos, and biopesticide spinosad. However, further studies are required to extract at the ultrastructural level the toxic effects of organophosphate insecticide Triazophos, biopesticide spinosad, and synthetic pyrethroid insecticide cypermethrin on *Branchiura sowerbyi* and to mitigate its toxicity using the appropriate plant extract.

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