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Assessment of Sesame Oil Toxicity on *Mesocyclops pehpeiensis*: acute and chronic effects, Immobilization test, Fecundity, and Protein expression studies

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Abstract

This study assessed the toxicity of sesame oil on *Mesocyclops pehpeiensis*, a common zooplankton species found in Uyyankondan River. The research encompassed acute and chronic toxicity tests, fecundity analysis, immobilization tests, and protein expression studies. The acute toxicity tests revealed concentration-dependent mortality in both nauplii and adults, with significant mortality observed at higher concentrations. Chronic exposure resulted in decreased survival rates and fecundity, indicating adverse effects on population dynamics. Immobilization tests showed significant effects of sesame oil on the mobility of adult copepods. Furthermore, protein expression analysis suggested potential adaptations to oil exposure. The findings highlight the ecological implications of sesame oil contamination on aquatic ecosystems and provide insights into copepod responses to oil pollutants.

Key words : *Mesocyclops pehpeiensis,* toxicity tests, fecundity analysis, immobilization tests.

Plankton, derived from the Greek words meaning "animal" and "floating" or "drifting," encompasses a diverse array of organisms crucial to understanding aquatic ecosystems. Among these, zooplankton, including copepods, play essential roles in marine environments. Copepods, the largest class of crustaceans, bridge the gap between phytoplankton and higher trophic levels, contributing significantly to energy transfer in aquatic food webs. Zooplankton, including copepods, are primary consumers in aquatic

ecosystems, feeding on phytoplankton and bacteria[1]. They serve as a vital source of nutrition for higher animals such as fish, especially during their larval stages. The distribution and abundance of zooplankton, particularly copepods, impact pelagic fisheries as they are preferred spawning sites for fish due to the abundance of planktonic food. Furthermore, copepods are integral to marine environmental science due to their ability to accumulate pollutants and function as indicators of environmental health. They are sensitive to changes in environmental variables such as temperature, salinity, and nutrient levels, making them valuable markers for assessing ecosystem integrity and responding to climate-driven changes. Bioluminescence, a fascinating phenomenon exhibited by copepods, serves multiple functions including communication, predator avoidance, and camouflage. Additionally, copepods contribute to the oceanic carbon pump, sequestering carbon through their feeding activities and facilitating its transport to deeper ocean layers[4].

Despite their ecological importance, copepod populations are vulnerable to various pollutants, including pharmaceuticals, nano plastics, and agricultural chemicals. These contaminants can negatively impact copepod reproduction, development, and behaviour, potentially disrupting marine food webs and ecosystem dynamics. Given the critical role of copepods in aquatic ecosystems and their susceptibility to pollutants, the present study aims to examine the survival, toxicity tolerance, reproductive capabilities and protein expression of *Mesocyclops pehpeiensis*, a species of copepod, when exposed to sesame oil. Understanding the effects of sesame oil on copepod survival and reproduction will provide insights into its potential impact on aquatic ecosystems and help inform better management practices for preserving marine environments. This study will also investigate the mobilization and life cycle of *Mesocyclops pehpeiensis* through fecundity tests and immobilization tests on ovigerous females and adults, contributing to our understanding of copepod ecology and behaviour.

Sample Collection and Culture Maintenance[11]

Samples were collected from sewage water in two distinct regions of the Uyyankondan River. After removing solid material with a filter, zooplankton were kept in de-chlorinated freshwater. *Mesocyclops pehpeiensis* was cultured in a lab setting with a pH of 7 and a temperature of 27°C. Spirulina was provided as feed once a day, and de-chlorinated freshwater was refilled every two days to maintain the culture.

Reagents :

Sesame oil ($C_7H_6O_3$) was used in this study. Its density at 25°C is 0.917 g/mL. Sesame oil contains omega-3, omega-6, and omega-9 fatty acids in balanced proportions.

Toxicity Tests :

Acute Toxicity : Ten nauplii and adults were exposed to different concentrations of sesame oil for 120 hours. Mortality rates were recorded daily under a light microscope.

Chronic Toxicity : Ten nauplii and adults were exposed to sesame oil for 120 hours. Mortality rates were recorded daily under a light microscope.

Immobilization and Fecundity Test :

Immobilisation Test: Organisms were incubated, and immobilization was observed after 2 and 4 days. Nauplii mortality was also observed for 24 and 48 hours.

Fecundity Test : Ovigerous females were exposed to different concentrations of sesame oil, and the number of nauplii produced was counted over 96 hours.

Protein expression study :

Protein samples were purified using RIPA lysis buffer and quantified using the Bradford assay. SDS-PAGE was performed to study protein expression.

Statistical Analysis : Data were analysed using the SPSS package.

The microscopic analysis revealed the presence of various organisms in the Uyyankondan River water, including copepods like *Mesocyclops pehpeiensis* (Fig. 1). Taxonomic identification placed *Mesocyclopspehpeiensis* within the class Copepoda, order Cyclopoida, family Cyclopidae, and genus *Mesocyclops*.

Figure 1. Photograph of the copepod *Mesocyclops pehpeiensis*

Nauplii and adults of *Mesocyclops pehpeiensis* were exposed to varying concentrations of sesame oil. No significant mortality was observed in the control group. Increasing concentrations of sesame oil led to higher mortality rates in both nauplii and adults, indicating dose-dependent toxicity (Table-1 and 2).

Table-1. Tabulation of acute toxicity test on nauplii of *Mesocyclops pehpeiensis*

Concentration of sesame oil induced	No. of mortality	No. of survival
Control	-	10
100ppm	-	10
500ppm	2	8

Table-2. Tabulation of acute toxicity test on adult of *Mesocyclops pehpeiensis*

Concentration of sesame oil induced	No. of mortality	No. of survival
Control	-	10
500ppm	-	10
1000ppm	2	8
2000ppm	3	7

Chronic exposure to sesame oil over 15 days resulted in increasing mortality rates in both nauplii and adults. Complete mortality was observed in some groups exposed to higher concentrations of sesame oil.

The acute and chronic toxicity tests demonstrated the adverse effects of sesame oil on *Mesocyclops pehpeiensis*, with increasing mortality rates observed with higher concentrations. The results of the acute toxicity

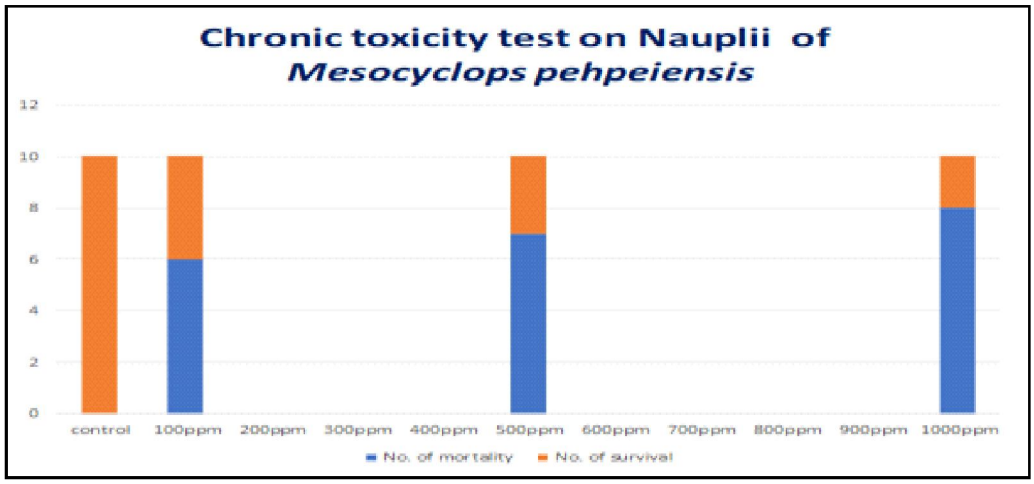

Figure 2. Graphical representation of chronic toxicity on nauplii

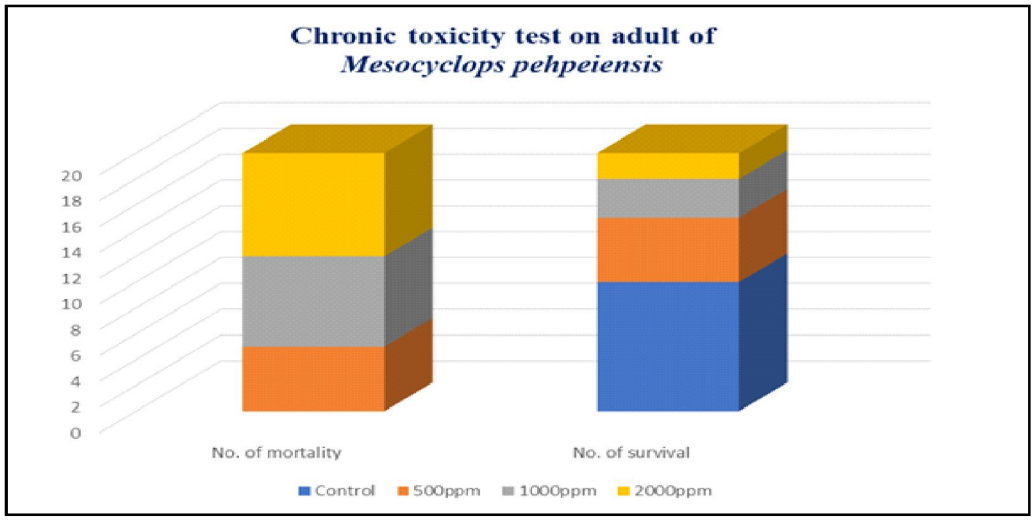

Figure 3. Graphical representation of chronic toxicity of adult

test demonstrated that exposure to sesame oil led to dose-dependent mortality in both nauplii and adult *Mesocyclops pehpeiensis*. The absence of significant mortality in the control group validates the toxicity observed in the treated groups. The nauplii were found to be more sensitive to sesame oil exposure compared to adults, as evidenced by higher mortality rates at lower concentrations. This sensitivity could be attributed to their smaller size and developing physiological systems, which may make them more susceptible to environmental stressors. In the chronic toxicity test, continuous exposure to sesame oil over 15 days resulted in increasing mortality rates in both nauplii and adult *Mesocyclops pehpeiensis*. Complete mortality was observed in some groups exposed to higher concentrations

of sesame oil, indicating a prolonged and cumulative toxic effect on the copepod population. The gradual decrease in survival rates over time suggests that the toxicity of sesame oil persists and accumulates in the aquatic environment, posing a significant threat to copepod populations and potentially disrupting the aquatic food chain. The results of the acute toxicity test demonstrated that exposure to sesame oil led to dose-dependent mortality in both nauplii and adult *Mesocyclops pehpeiensis*. The absence of significant mortality in the control group validates the toxicity observed in the treated groups[13]. The nauplii were found to be more sensitive to sesame oil exposure compared to adults, as evidenced by higher mortality rates at lower concentrations. This sensitivity could be attributed to their smaller size and developing physiological systems, which may make them more susceptible to environmental stressors[3]. In the chronic toxicity test, continuous exposure to sesame oil over 15 days resulted in increasing mortality rates in both nauplii and adult Mesocyclops pehpeiensis. Complete mortality was observed in some groups exposed to higher concentrations of sesame oil, indicating a prolonged and cumulative toxic effect on the copepod population[10]. The gradual decrease in survival rates over time suggests that the toxicity of sesame oil persists and accumulates in the aquatic environment, posing a significant threat to copepod populations and potentially disrupting the aquatic food chain[12].

The fecundity test showed a decrease in the ratio of adult females to males as the sesame oil concentration increased. Survival rates decreased with increasing sesame oil concentration, affecting the differentiation of nauplii into female adults.

The fecundity test revealed a decline in reproductive capacity under sesame oil exposure. The fecundity test revealed adverse effects of sesame oil on the reproductive capacity of *Mesocyclops pehpeiensis*. As the concentration of sesame oil increased, there was a decrease in the ratio of adult females to males, indicating a disruption in the normal reproductive cycle of the copepods. The decline in survival rates of nauplii under sesame oil exposure further emphasizes the detrimental impact on population growth and sustainability. The fecundity test revealed adverse effects of sesame oil on the reproductive capacity of *Mesocyclops pehpeiensis*. As the concentration of sesame oil increased, there was a decrease in the ratio of adult females to males, indicating a disruption in the normal reproductive cycle of the copepods[9]. The decline in survival rates of nauplii under sesame oil exposure further emphasizes the detrimental impact on population growth and sustainability[5].

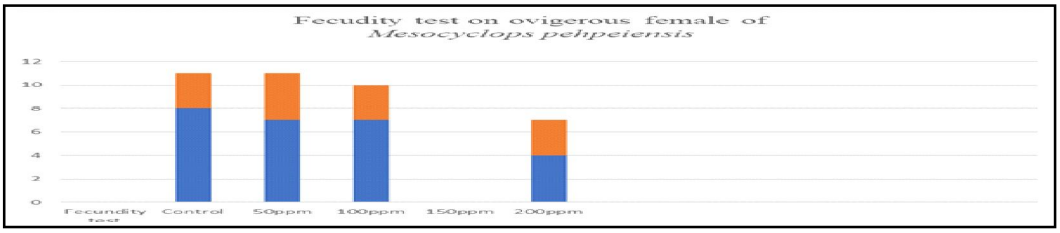

Figure 4. Fecundity test on ovigerous female of *Mesocyclops pehpeiensis*

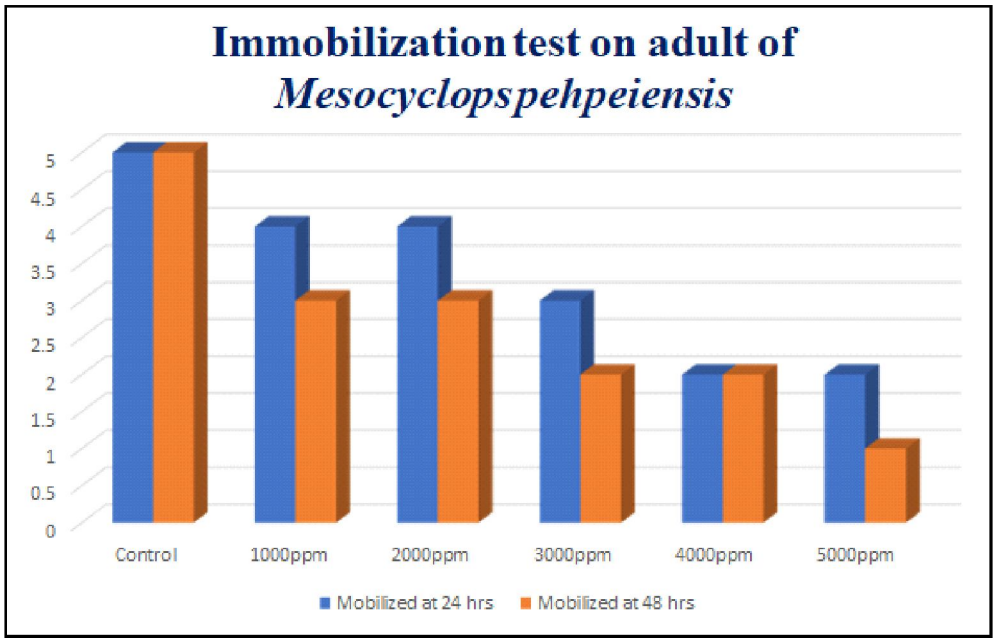

Figure 5. Immobilization test on adults of *Mesocyclops pehpeiensis*

Exposure to sesame oil led to increased immobilization of adults of Mesocyclops pehpeiensis, with higher concentrations causing higher immobilization rates. The test also indicated mortality in different concentrations, with higher concentrations leading to more immobilization and mortality.

The immobilization test showed the sensitivity of *Mesocyclops pehpeiensis* to sesame oil, with significant immobilization observed even at lower concentrations. The immobilization test demonstrated the acute toxic effects of sesame oil on adult *Mesocyclops pehpeiensis*, with higher concentrations leading to increased immobilization rates. The observed immobilization can be attributed to the neurotoxic effects of the oil, which interfere with the normal locomotion and behavior of the copepods. The mortality observed in the test further underscores the severity of the toxic effects, with higher concentrations resulting in significant mortality within 48 hours. The immobilization test demonstrated the acute toxic effects of sesame oil on adult Mesocyclops pehpeiensis, with higher concentrations leading to increased immobilization rates. The observed immobilization can be attributed to the neurotoxic effects of the oil, which interfere with the normal locomotion and behavior of the copepods[2]. The mortality observed in the test further underscores the severity of the toxic effects, with higher concentrations resulting in significant mortality within 48 hours[8].

Protein expression analysis showed upregulation of proteins in the treated group, particularly in the 35-48 kDa range, indicating a potential adaptive response to oil toxicity.

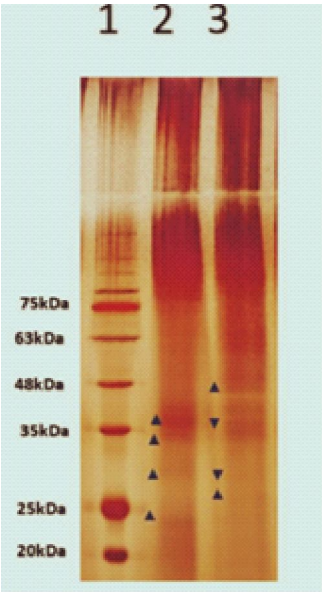

Figure 6. SDS PAGE analysis of *Mesocyclops pehpeiensis,* Lane 1: Protein marker ladder; Lane 2: Control (without oil treatment); Lane 3: Treated-1000 ppm

The upregulation of certain proteins in the treated group suggests a potential adaptive response to mitigate the toxic effects of sesame oil. The upregulation of certain proteins in the treated group, particularly in the 35-48 kDa range, suggests a potential adaptive response of Mesocyclops pehpeiensis to mitigate the toxic effects of sesame oil. These proteins may play a role in detoxification mechanisms or cellular repair processes, allowing the copepods to cope with the stress induced by sesame oil exposure. The upregulation of certain proteins in the treated group, particularly in the 35-48 kDa range, suggests a potential adaptive response of *Mesocyclops pehpeiensis* to mitigate the toxic effects of sesame oil[7]. These proteins may play a role in detoxification mechanisms or cellular repair processes, allowing the copepods to cope with the stress induced by sesame oil exposure[6].

These findings underscore the importance of assessing the impact of pollutants on aquatic organisms and highlight the vulnerability of copepods like Mesocyclops pehpeiensis to environmental stressors like sesame oil contamination. The findings of this study highlight the vulnerability of Mesocyclops pehpeiensis to sesame oil contamination and underscore the importance of assessing the impact of pollutants on aquatic organisms. The observed acute and chronic toxicity, as well as adverse effects on reproduction and protein expression, emphasize the need for effective monitoring and management strategies to protect aquatic ecosystems from oil pollution. Additionally, the results provide valuable insights into the potential ecological consequences of sesame oil contamination on copepod populations and aquatic food webs. Further research is warranted to elucidate the underlying mechanisms of toxicity and to develop mitigation measures to minimize the adverse effects on aquatic organisms and ecosystems. The findings of this study highlight the vulnerability of Mesocyclops pehpeiensis to sesame oil contamination and underscore the importance of assessing the impact of pollutants on aquatic organisms[14]. The observed acute and chronic toxicity, as well as adverse effects on reproduction and protein expression, emphasize the need for effective monitoring and management strategies to protect aquatic ecosystems from oil pollution[15]. Additionally, the results provide valuable insights into the potential ecological consequences of sesame oil contamination on copepod

populations and aquatic food webs. Further research is warranted to elucidate the underlying mechanisms of toxicity and to develop mitigation measures to minimize the adverse effects on aquatic organisms and ecosystems.

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