Culturing Daphnia Species and Evaluation of toxicity of Acetamiprid

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Abstract:

This study investigated the acute and chronic toxicity of Acetamiprid on Daphnia spp. under controlled laboratory conditions. Results showed a clear concentration- and time-dependent increase in mortality, with a 24-hour LC_{50} of 0.52 mg/L and a more sensitive 96-hour LC_{50} of 0.28 mg/L, while both NOEC and LOEC were observed at 0.2 mg/L. Higher concentrations caused severe behavioral and morphological changes, including reduced swimming, antennal damage, immobilization, and increased mortality. Overall, the findings demonstrate that Acetamiprid poses significant toxic risks to freshwater invertebrates and may threaten aquatic ecosystems.

Introduction:

Daphnia spp. are freshwater cladoceran zooplankton belonging to the family Daphniidae and are commonly known as “water fleas” due to their characteristic swimming movement. They are important components of freshwater ecosystems, acting as primary consumers and linking phytoplankton with higher trophic organisms. Due to their short life cycle, parthenogenetic reproduction, transparent body, easy laboratory maintenance, and sensitivity to environmental changes, Daphnia spp. are widely used as standard model organisms in ecotoxicological research and water quality assessment.

Increasing pesticide contamination in aquatic environments has become a major concern due to agricultural runoff, leaching, and other pathways of chemical entry into water bodies. Pesticides can negatively affect non-target aquatic organisms, especially zooplankton species that play vital roles in maintaining ecosystem balance. Daphnia spp. are highly sensitive to pollutants, and changes in their survival, mobility, growth, and reproduction serve as important indicators of environmental toxicity.

Acetamiprid is a neonicotinoid insecticide widely used in agriculture for controlling sucking pests such as aphids, whiteflies, and thrips. Due to its high water solubility, it can enter freshwater ecosystems and cause harmful effects on non-target organisms including Daphnia spp. Exposure to acetamiprid may lead to acute and sub-lethal toxic effects such as immobilization, mortality, oxidative stress, and reproductive changes. Therefore, studying the toxicity of acetamiprid using Daphnia spp. is essential for evaluating ecological risks and understanding the impact of pesticides on freshwater biodiversity.

Materials and Methods

The study was conducted using Daphnia spp. collected from a freshwater pond and cultured under controlled laboratory conditions. Healthy individuals were identified using morphological characteristics and maintained in filtered pond water mixed with ASTM

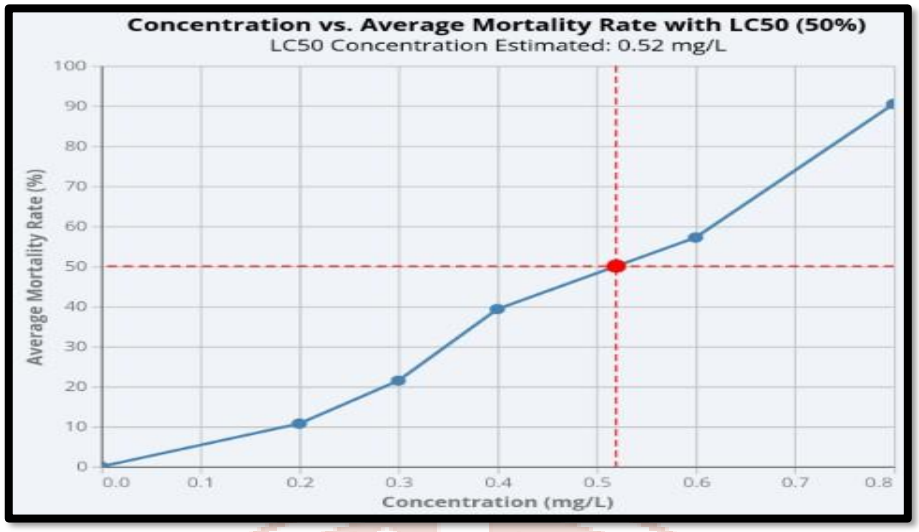
reconstituted hard water. The culture conditions were maintained at suitable temperature (20–25°C), pH (7.0–8.0), dissolved oxygen, and a 16:8 hour light-dark cycle. Daphnia were provided with algal food (Spirulina) for proper growth and reproduction. Water quality parameters including temperature, pH, TDS, TSS, salinity, dissolved oxygen, BOD, hardness, alkalinity, and acidity were analysed before toxicity experiments.

Acetamiprid 20% SP (FLYKING), a neonicotinoid insecticide, was used as the test chemical for toxicity evaluation. Acute toxicity tests were performed following OECD Guideline 202 using less than 24-hour-old neonates of Daphnia spp. exposed to different concentrations of acetamiprid for 48 hours, and immobilization and mortality were recorded to determine EC_{50} values. Chronic toxicity tests were conducted according to OECD Guideline 211 to assess long-term effects on survival and reproduction. Parameters such as mortality, growth, brood production, NOEC, and LOEC values were evaluated using appropriate statistical analysis.

Results and Discussion

Fig:1 Daphnia spp. Culture media M4 and M7 ASTM

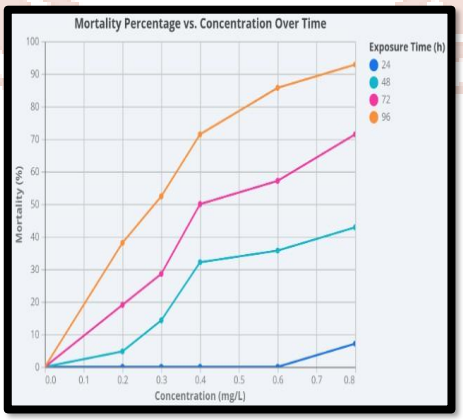
The laboratory culture of Daphnia spp. was successfully established under controlled conditions. The organisms showed normal morphology, active swimming behaviour, and continuous reproduction. Suitable water quality parameters (temperature 24.35°C, pH 7.4, and DO 4.0 mg/L) supported healthy growth and ensured reliable toxicity assessment.



Graph: 2 Acute (24 h) LC50:0.52 mg/L

Acute toxicity evaluation of Acetamiprid showed a clear concentration-dependent increase in mortality of Daphnia spp. Mortality increased from 10.71% at 0.2 mg/L to 90.48% at 0.8 mg/L, with an estimated LC_{50} value of 0.52 mg/L after 24 hours.

Graph:3 Chronic (96 h) LC50 :0.28 mg/L



Graph:5 Time-Dependent Mortality

Chronic exposure resulted in higher toxic effects over time. After 96 hours, mortality reached 92.85% at 0.8 mg/L concentration, and the LC_{50} value decreased to 0.28 mg/L, indicating cumulative toxicity of Acetamiprid. The NOEC and LOEC values were recorded at 0.2 mg/L.

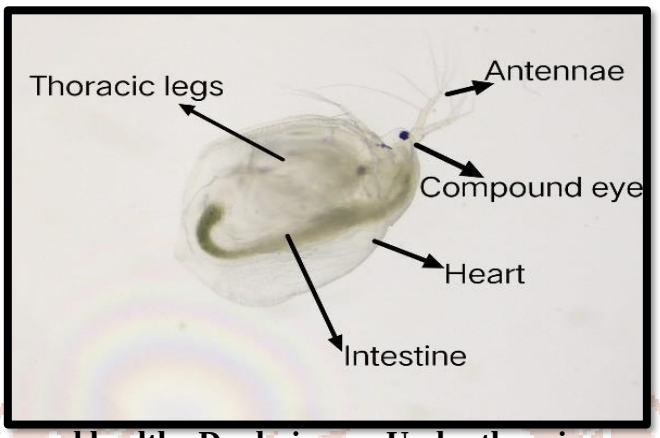


Fig:6 Normal healthy Daphnia spp. Under the microscope 10 X.

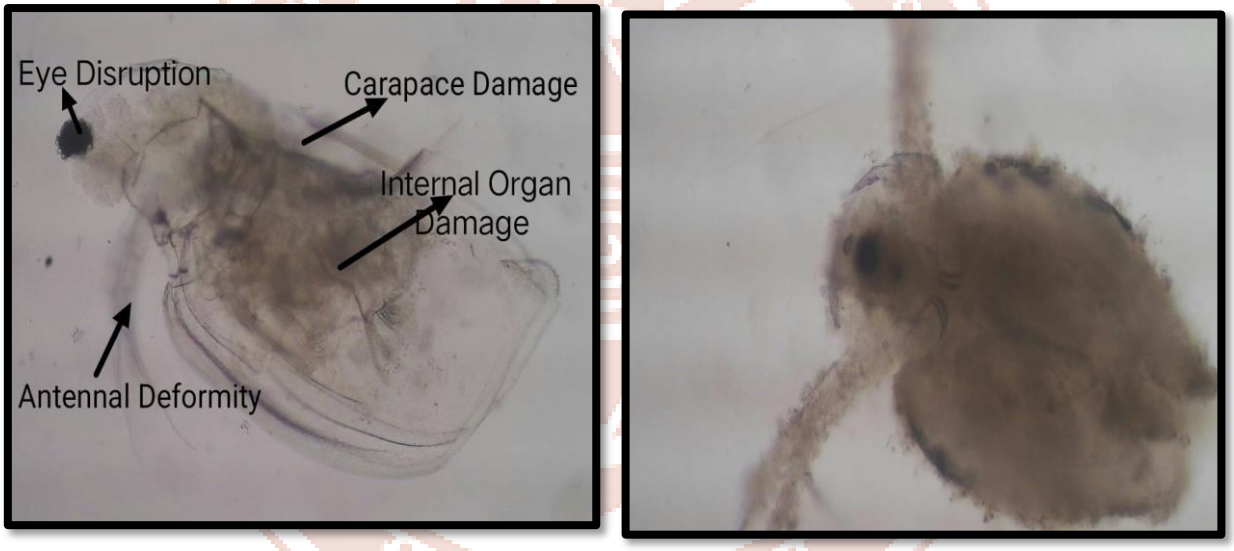


Fig:9 Daphnia spp. Under microscope at 0.8 mg/L

Microscopic observations revealed concentration-dependent behavioural and morphological changes. Low concentrations caused reduced swimming activity, while higher concentrations resulted in immobilization, loss of balance, inactive antennae, and mortality. These findings confirm the high sensitivity of Daphnia spp. as a bioindicator for pesticide toxicity assessment.

Conclusion

This study evaluated the toxic effects of the neonicotinoid pesticide Acetamiprid on Daphnia spp., a widely used bioindicator in aquatic ecotoxicology. The successful laboratory culture under controlled conditions confirmed that Daphnia is a suitable model organism for assessing pesticide toxicity. The results showed a clear concentration- and time-dependent

increase in mortality, with acute LC_{50} of 0.52 mg/L and chronic LC_{50} of 0.28 mg/L, indicating increased toxicity during prolonged exposure.

Acetamiprid exposure also caused significant behavioural and physiological changes, including reduced swimming activity, loss of balance, antennal inactivity, immobilization, and mortality at higher concentrations. The findings demonstrate that even low concentrations of Acetamiprid can negatively affect non-target aquatic organisms. Therefore, proper pesticide management and continuous monitoring are essential to protect freshwater biodiversity and ecosystem stability.

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