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Effect of Coragen pesticide on protein content of *Gambusia affinis*

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Abstract
Fish are widely recognized as bioindicators of aquatic pollution, reflecting the impact of pesticides on ecosystem integrity and human health due to their position in the food chain. Freshwater ecosystems, including lentic and lotic systems, sustain diverse fish populations whose survival is influenced by physicochemical parameters and environmental stressors. The mosquitofish (*Gambusia affinis*), owing to its wide distribution, adaptability, and high reproductive capacity, serves as an effective model organism in toxicological studies. In the present study, the LC_{50} value of the tested pesticide was determined to be 3 ppm, indicating moderate acute toxicity.
Biochemical analysis revealed a concentration- and time-dependent decline in protein content. After 24 hours of exposure, protein levels decreased remarkably from the control value (172 µg; OD 1.9) to 2.7 µg (OD 0.03) at 2.5 ppm. A further reduction was observed after 48 hours, with protein content reaching 1.8 µg (OD 0.02) at the same concentration. These findings suggest that the pesticide exerts significant toxic effects by disrupting protein metabolism, possibly through inhibition of synthesis or enhanced degradation, thereby indicating potential ecological risks in aquatic environments.

Keywords: *Gambusia affinis*, Coragen pesticide, LC_{50}, protein

Introduction
Aquatic ecosystems, including lentic and lotic environments, are essential for maintaining ecological integrity and biodiversity. Fish, as key components of these systems, serve as sensitive bioindicators of environmental contamination due to their continuous exposure to pollutants and their position in the food web. Variations in physicochemical and biological parameters of aquatic habitats can significantly influence fish health, making them reliable models for ecotoxicological assessments.
Fishes are particularly sensitive to any change in physicochemical as well as biological characteristics of aquatic bodies. The toxic chemicals in aquatic environment are proved to be dangerous for the survival of fish (Caldas *et al*., 1999; Lamai *et al*., 1999) [4, 6] Water pollution is the cause of death of several interdependent aquatic forms of life and also a source of bio-magnification of persistent pesticides. This can result in local effect on environment and mortality of fish. The extensive use of pesticides in agriculture and public health programs has resulted in widespread contamination of aquatic ecosystems through runoff, leaching, and atmospheric deposition. Among these, organophosphorus compounds such as dichlorvos (DDVP) and insecticides like chlorantraniliprole are known to induce toxicity by disrupting essential physiological and biochemical processes, including inhibition of acetylcholinesterase activity, leading to impaired neural function.
A very small amount of total pesticides is in fact effective in killing or controlling target pests, while the remaining large amount is released into the environment, including aquatic ecosystems, where it negatively impacts non-target species (Tudi *et al*., 2021[12]; Özkara *et al*., 2016; Raut and Kurhe, 2022) [9]. There is overwhelming evidence depicting the adverse effects of pesticides on aquatic ecosystems (Barlas 1999; Aktar *et al*., 2009; Raut and Kurhe, 2022) [1, 2, 9].
Gambusia affinis (mosquitofish), a widely distributed lar*vivo*rous freshwater species, is extensively used in toxicological studies owing to its high adaptability, rapid reproductive rate, and sensitivity to environmental stressors. Its biological characteristics, including a short life cycle and ease of laboratory maintenance, make it an ideal model for assessing pollutant-induced toxicity in aquatic systems.
Among biochemicals, protein content is a critical measure of metabolic status, growth, and physiological stress in fish. Alterations in protein metabolism reflect the sublethal effects of toxic exposure.
Hence, the present study evaluates pesticide-induced toxicity of Coragen in *Gambusia affinis*, with emphasis on LC_{50} determination and changes in protein content, to elucidate the ecological risks associated with pesticide contamination in aquatic environments.

Materials and Methods
The freshwater fish *Gambusia affinis* (mosquitofish) was used as the experimental organism, and the insecticide Coragen (chlorantraniliprole 18.5% SC) was selected for toxicity assessment. Standard laboratory glassware equipment and chemicals were used in the study. Specimens of *G. affinis* were collected from a pond located within the campus of Sangamner Nagarpalika Arts, D. J. M. Commerce and B.N.S. Science College (Autonomous), Sangamner, Ahilyanagar, Maharashtra, India, using a fishing net, randomly selected and transported to the laboratory in plastic containers. The fish were acclimatized under laboratory conditions for two weeks prior to experimentation, and individuals of approximately equal

size were selected. Prior to maintenance, fish were disinfected with 0.1% potassium permanganate ($KMnO_4$) solution to reduce dermal infections and disease outbreaks. During acclimatization, fish were kept in aquaria containing dechlorinated pond water, which was renewed every 24 hours, and were fed with zooplankton from pond water; species identification was confirmed using standard taxonomic keys. A static bioassay was performed to determine the 96-hour LC_{50} of Coragen, with fish starved for 24 hours before exposure. The experiment consisted of six groups (10 fish per 1 L of pond water), where one group served as control and the remaining groups were exposed to graded concentrations of Coragen (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm), and mortality was recorded at regular intervals; LC_{50} values were calculated using Finney's probit analysis method. Based on LC_{50} results, a sublethal concentration was selected for biochemical analysis, where fish were divided into control and treated groups and exposed for 24 and 48 hours; thereafter, fish were sacrificed and whole-body tissues were collected for analysis. Protein content was estimated using the Lowry method, absorbance was measured using a colorimeter, and protein concentration was determined using a bovine serum albumin standard curve.

Results

Table 1: % Mortality against Log Concentration

Sr. No.	Conc. of Pesticide in ppm	Log of conc.	No. of fishes exposed	No. of fishes alive	No. of fishes dead	Percent mortality (%)	Probit mortality
1	Control	0.0	10	10	00	00	-
2	1	0.0	10	10	1	10	3.72
3	2	0.30	10	9	2	20	4.16
4	3	0.47	10	5	5	50	5.00
5	4	0.60	10	02	8	80	5.84
6	5	0.69	10	00	10	100	8.09

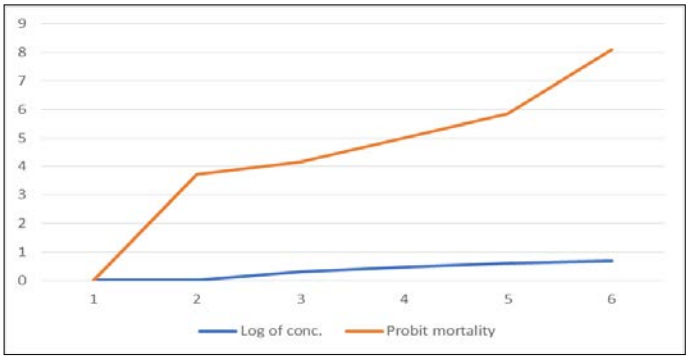

Fig 1: Probit mortality against Log Concentration

The table and figure number 1 shows a clear dose-dependent increase in mortality of *Gambusia affinis* with increasing concentrations of Coragen, where percent mortality rises from 10% at 1 ppm to 100% at 5 ppm, with corresponding increases in probit mortality values, indicating higher toxicity at elevated pesticide concentrations.

Table 2: Observation table for Protein estimation of 24 hrs. Fish exposed to pesticide

	Volume of standard BSA (ml)	Volume of distilled water (ml)	Conc. of protein (µg)	Volume of reagent C (ml)		Volume of reagent D (ml)		Optical Density (A660)
Control	1.0	0.0	172	5	Incubate at room Temp. for 10 min	0.5	Incubate at dark room Temp. for 30 min	1.9
0.5ppm	1.0	0.0	20	5		0.5		0.23
1ppm	1.0	0.0	18	5		0.5		0.20
1.5ppm	1.0	0.0	8.1	5		0.5		0.09
2ppm	1.0	0.0	5.4	5		0.5		0.06
2.5ppm	1.0	0.0	2.7	5		0.5		0.03

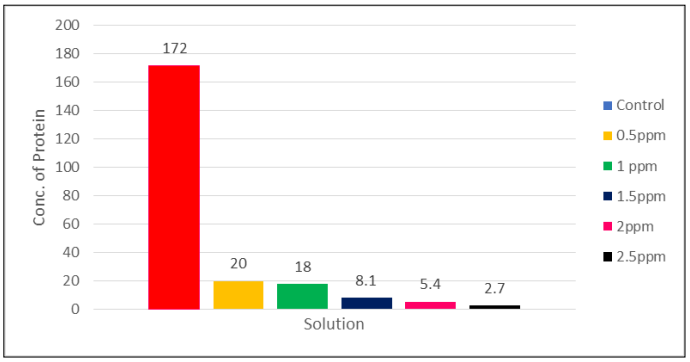

Fig 2: Graph of Conc. of Protein vs Conc. of Solution

The table and figure number 2 shows the effect of different concentrations of Coragen on protein content in *Gambusia affinis*, where the control group exhibits the highest protein level (172 µg, OD 1.9), while increasing pesticide concentrations (0.5–2.5 ppm) result in a progressive decline in both protein content and optical density (A660), indicating a dose-dependent reduction in protein due to pesticide exposure.

Table 3: Observation table for Protein estimation of 48 hrs. fish exposed to pesticide

	Volume of standard BSA (ml)	**Volume of distilled water (ml)**	**Conc. of protein (µg)**	**Volume of reagent C (ml)**	**Incubate at room Temp. for 10 min**	**Volume of reagent D (ml)**	**Incubate at dark room Temp. for 30 min**	**Optical Density (A660)**
Control	1.0	0.0	172	5		0.5		1.9
0.5ppm	1.0	0.0	14.5	5		0.5		0.16
1ppm	1.0	0.0	12.7	5		0.5		0.14
1.5ppm	1.0	0.0	10.0	5		0.5		0.11
2ppm	1.0	0.0	5.4	5		0.5		0.06
2.5ppm	1.0	0.0	1.8	5		0.5		0.02

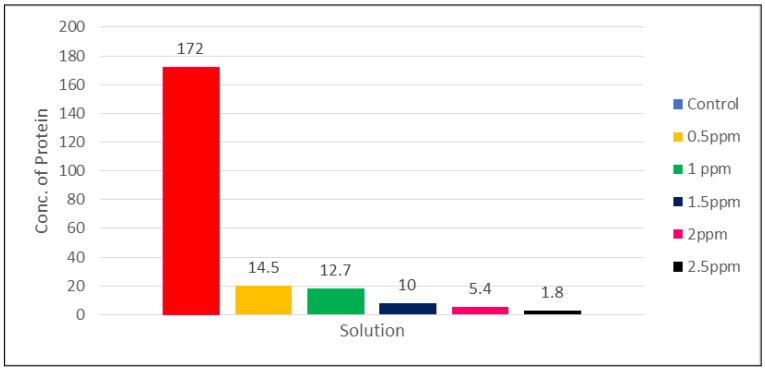

Fig 3: Graph of Conc. of Protein vs Conc. of Solution

The table and figure number 3 illustrates a dose-dependent decrease in protein content and optical density (A660) in *Gambusia affinis* exposed to increasing concentrations of Coragen (0.5–2.5 ppm), where the control group shows the highest protein level (172 µg, OD 1.9) and the treated groups exhibit progressively reduced protein values, indicating pesticide-induced inhibition of protein metabolism

Discussion

The present study demonstrated a significant, dose-dependent reduction in protein content in *Gambusia affinis* exposed to Coragen (chlorantraniliprole 18.5% SC). The biochemical analysis showed a progressive decline in protein levels from the control group (172 µg; OD 1.9) to the highest pesticide concentration (2.5 ppm; 1.8 µg; OD 0.02), indicating a marked disruption of protein metabolism under pesticide stress.

Similar findings have been reported by Verma and Rawat in 2017, who observed variations in protein and carbohydrate levels in ovarian tissues of fish exposed to chlorpyrifos, suggesting that pesticide exposure alters biochemical reserves in aquatic organisms. Proteins act as an alternative energy source under stress conditions when carbohydrate reserves are insufficient and are also essential in regulating intracellular and extracellular interactions, thereby maintaining physiological homeostasis.

Comparable results were reported by Revathi and Krishnamurthy, 2018 [10], who investigated the effects of chlorpyrifos on *Channa striatus*. They observed a significant reduction in total protein content in liver, muscle, and gill tissues under sublethal and lethal exposures, with gill tissues showing the greatest decline compared to control. The authors attributed this decrease to enhanced proteolysis and metabolic stress induced by pesticide toxicity.

Kulkarni *et al*., 2015 [5], reported alterations in sodium and potassium levels in the haemolymph of the freshwater crab *Barytelphusa guerini* exposed to toxicants, indicating that pesticide exposure disrupts ionic balance and metabolic homeostasis. They further noted partial recovery in biochemical parameters upon supplementation with sulfur-containing amino acids such as methionine, suggesting the involvement of amino acid metabolism in stress adaptation.

Sujatha *et al*., 2013 [11], highlighted the nutritional importance of fish proteins, reporting that fish tissues are rich in essential amino acids, particularly lysine, which enhances their biological and nutritional value. This emphasizes that any reduction in protein content due to toxic exposure may significantly affect the physiological and ecological quality of fish populations.

Similar biochemical alterations were also reported by Pawar *et al*., 2016 [8], who observed significant depletion in protein and glycogen levels in *Gambusia affinis* exposed to sublethal concentrations of pesticides. The authors suggested that such reductions are a consequence of increased metabolic demand under toxic stress, where energy is mobilized from protein and carbohydrate reserves to support detoxification and survival mechanisms.

Borgave *et al*., 2020 [3] worked on Toxicity of Coragen on Early Zebrafish Embryo. From their study they suggested that use of Coragen in the fields as it affects the aquatic vertebrates even at very low concentrations thus disturbing the aquatic ecosystem.

Overall, the observed decline in protein content in the present study may be attributed to enhanced proteolysis,

impaired protein synthesis, and increased energy demand required to counteract pesticide-induced stress. These biochemical disruptions indicate that Coragen exposure exerts significant metabolic stress on *Gambusia affinis*, thereby affecting its physiological stability and ecological fitness.

Conclusion

The present study shows that Coragen (chlorantraniliprole 18.5% SC) causes significant biochemical stress in *Gambusia affinis*, leading to a marked decrease in protein content and concentration-dependent mortality with an LC_{50} value of about 3 ppm. The reduction in protein levels from control to treated groups confirms pesticide-induced metabolic disruption, indicating that protein is a sensitive biomarker of toxicity. The study also highlights the role of *G. affinis* as a bioindicator species and emphasizes that pesticide exposure can impair physiological functions and threaten freshwater ecosystem balance, underscoring the need for regulated pesticide use.

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