

Identification of the Most Effective Dosage of Clove Oil Solution as an Alternative for Synthetic Anaesthetics on Zebrafish (*Danio rerio*)

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Abstract : Zebrafish (*Danio rerio*) in the family Cyprinidae, is a tropical freshwater fish widely used as a model organism in scientific research. Use of effective and economical anaesthetic is very important when handling fish. Clove oil (active ingredient: eugenol) was identified as a natural product which is safer and economical compared to synthetic chemicals like methanesulfonate (MS-222). Therefore, the aim of this study was to identify the most effective dosage of clove oil solution as an anaesthetic on mature Zebrafish. Clove oil solution was prepared by mixing pure clove oil with 94% ethanol at a ratio of 1:9 respectively. From that solution, different volumes were selected as (0.4 ml, 0.6 ml and 0.8 ml) and dissolved in one liter of conditioned water (dosages : 0.4 ml/L, 0.6 ml/L and 0.8 ml/L). Water quality parameters (pH, temperature and conductivity) were measured before and after adding clove oil solution. Mature Zebrafish with similar standard length (2.76 ± 0.1 cm) and weight (0.524 ± 0.1 g) were selected for this experiment. Time taken for loss of equilibrium (initiation phase) and complete loss of movements including opercular movement (anaesthetic phase) were measured. To detect the efficacy on anaesthetic recovery, time taken to begin opercular movements (initiation of recovery phase) until swimming (post anaesthetic phase) were observed. The results obtained were analyzed according to the analysis of variance (ANOVA) and Tukeys' method using SPSS version 17.0 at 95% confidence interval ($p < 0.5$). According to the results, there was no significant difference at the initiation phase of anaesthesia in all three doses though the time taken was varied from 0.14 to 0.41 minutes. Mean value of the time taken to complete the anaesthetic phase at 0.4 ml/L dosage was significantly different with 0.6 ml/L and 0.8 ml/L dosages independently ($p = 0.01$). There was no significant difference among recovery times at all dosages but 0.8 ml/L dosage took longer time compared to 0.6 ml/L dosage. The water quality parameters (pH and temperature) were stable throughout the experiment except conductivity, which increased with the higher dosage. In conclusion, the best dosage need to anaesthetize Zebrafish using clove oil solution was 0.6 ml/L due to its fast initiation of anaesthesia and quick recovery compared to the other two dosages. Therefore clove oil can be used as a good substitute for synthetic anaesthetics because of its efficacy at a lower dosage with higher safety at a low cost.

Keywords : anaesthetics, clove oil, zebrafish, Cyprinidae

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