

Table 4 Mortality outcomes at 4 000 mg L⁻¹

Extract	<i>C. gariepinus</i>	<i>O. niloticus</i>
<i>C. sinensis</i>	0%	0%
<i>C. aurantium</i>	100%	100%
<i>C. limon</i>	100%	100%

Twenty-four-hour post-exposure mortality rates (%) of *Clarias gariepinus* and *Oreochromis niloticus* following exposure to the maximum tested concentration (4 000 mg L⁻¹) of citrus leaf extracts.

In this study, the experimental unit was defined as the replicate tank rather than individual fish, as fish within the same tank were exposed simultaneously to identical environmental and treatment conditions. Treating individual fish as independent observations would violate the assumption of independence and lead to pseudoreplication. The use of tank-level replication is widely accepted in aquaculture experimentation and provides a statistically valid framework for detecting treatment-related differences in anesthetic response dynamics (Neiffer, 2021; Vergneau Grosset and Benedetti, 2022; Rodrigues Brandão et al., 2022)

3.4 Recovery time patterns across extracts and concentrations

Recovery times across extract types and concentration categories are summarised (Table 5). One way analysis of variance revealed significant differences among treatment groups ($F(6,14) = 8.63$, $p = 0.001$).

The analysis included only treatments in which recovery occurred, resulting in an unbalanced dataset due to the exclusion of high concentration treatments associated with complete mortality. Consequently, comparisons are limited to recoverable conditions.

Recovery time increased with extract potency. *Citrus limon* was associated with the longest recovery periods, followed by *Citrus aurantium*, while *Citrus sinensis* consistently showed the shortest recovery times. Post hoc comparisons using Tukey's test were conducted to facilitate interpretation; however, given the limited number of replicates, these comparisons should be regarded as exploratory.

Table 5 Recovery time across citrus extracts and concentrations

Extract	Dosage Category	Recovery Time (min)
Sweet Orange	Low	5.1 ± 0.38 ^a
	Moderate	10.9 ± 0.55 ^b
	High	14.8 ± 0.72 ^c
Sour Orange	Low	8.7 ± 0.47 ^b
	Moderate	14.7 ± 0.81 ^c
Lemon	Low	9.1 ± 0.44 ^b
	Moderate	16.2 ± 0.93 ^d

3.5 Comparative anaesthetic performance of citrus extracts

Comparative anaesthetic performance across the three extracts is summarised in Table 6. This assessment integrates behavioural observations, recovery patterns, and mortality outcomes. *Citrus sinensis* exhibited relatively low anaesthetic potency but a wide safety margin. *Citrus aurantium* demonstrated moderate potency with a narrower safety margin, while *Citrus limon* showed high potency but was associated with a very limited safety margin. This comparison is qualitative and was not subjected to statistical testing. The findings suggest a trade off between anaesthetic efficacy and safety among the extracts.

Table 6 Comparative anaesthetic performance

Extract	Relative Potency	Safety Margin
Sweet Orange	Low	Wide
Sour Orange	Moderate	Narrow
Lemon	High	Very narrow