**Application**

The present study validates the antioxidant potential of *Ocimum gratissimum* essential oil in aquatic organisms and it shows its potential use in the aquaculture sector. The *O. gratissimum* essential oil enhances prawns’ innate immune system by stimulating the non-enzymatic antioxidant defence. Therefore, the *O. gratissimum* essential oil contributes to the health and welfare of farmed prawns.

**Introduction**

Freshwater prawn farming is among the main aquaculture activities. In South America, the *Macrobrachium amazonicum* is the major native species with aquaculture potential. It presents desirable performance and biological characteristics, including robustness, year-round breeding, and disease resistance. Although native species present lower environmental risks, farm confinement can cause animal stress and depress the immune system, leading to prawns being more susceptible to diseases.

As a result, outbreaks may occur due to stress and, consequently, increasing the use of antibiotics. Antibiotics may lead to antimicrobial resistance and accumulation of toxic residues in the environment (Hassaan et al., 2021). Therefore, it is essential to search for sustainable alternatives to minimize the use of antibiotics. Among the promising phytotherapics as alternatives to synthetic drugs are essential oils. Current studies are focused on evaluating the analgesic properties of essential oils, and most of them are done with fish. Studies with essential oils and decapod crustaceans are still very scarce.

Therefore, this research aimed to evaluate the antioxidant capacity of the clove basil (*Ocimum gratissimum)* essential oil (OG-EO) in the post-larvae (PL) of *M. amazonicum*. To date, there are no studies evaluating the potential antioxidant activity of OG-EO in *M. amazonicum*, which emphasizes the originality of the research.

**Material and methods**

To evaluate the antioxidant potential of EO-OG, a total of 360 PL of *M. amazonicum* (average weight 0.054 ± 0.010 g) was used (15 prawns per experimental unit). Prawns were obtained from the larviculture system of the Shrimp Culture Laboratory, Federal University of Paraná, Brazil. Different dietary inclusion levels of OG-EO were tested: 0%, 1%, 2%, and 3%. The experimental design consisted of 24 tanks (experimental units; *n* = 6) of 40 L (useful capacity), maintained with continuous aeration and constant water recirculation in a completely randomized design. Prawns were fed until apparent satiety four times a day (03:00; 09:00; 14:00; and 18:00). During the feeding trial, water temperature, dissolved oxygen and pH were analysed daily, while nitrite and total ammonia were measured weekly. For the analysis of prawn performance, the final weight, total length, biomass, biomass gain, survival, and feed conversion were evaluated.

To evaluate the biochemical parameters, the hepatopancreas of 32 PLs (*n* = 8 per experimental group) were sampled, immediately stored in sterile 2 mL microtubes in liquid nitrogen, and sent to the Laboratory of Biochemistry and Genetics of the Federal University of Southern Border, Brazil. Tissues were then processed and used to evaluate the hepatopancreas' antioxidant capacity. All data obtained were evaluated for normality and homoscedasticity. Data when then subjected to one-way ANOVA followed by a mean comparison test (Tukey) at the 5% level of significance.

**Results**

All growth parameters analysed were similar among the experimental groups (Table 1). This result corroborated previous studies that showed that dietary *Lippia alba* essential oil (1% and 2%) has no impact on *M. rosenbergii* performance (De Souza et al. 2024).

**Table 1.** Growth performance of *Macrobrachium amazonicum* post-larvae fed diets supplemented with different levels of *Ocimum gratissimum* essential oil.

|  |  |
| --- | --- |
| **Growth parameters** | **Experimental groups (g OG-EO kg diet −1)** |
| **0%** | **1%** | **2%** | **3%** | ***p*-value** |
| **Weight (g)** | 0.32 ± 0.09 | 0.34 ± 0.08 | 0.31 ± 0.09 | 0.31 ± 0.07 | *0.1734* |
| **Total length (cm)** | 3.2 ± 0.38 | 3.3 ± 0.41 | 3.2 ± 0.44 | 3.1 ± 0.32 | *0.0705* |
| **Survival (%)** | 80 ± 12.15 | 80 ± 3.35 | 86 ± 5.40 | 80 ± 8.30 | *0.4371* |
| **Growth rate (g)** | 1.5 ± 0.15 | 1.5 ± 0.09 | 1.4 ± 0.07 | 1.2 ± 0.17 | *0.2740* |
| **Feed conversion** | 1.5 ± 0.30 | 1.4 ± 0.17 | 1.6 ± 0.16 | 2.1 ± 0.45 | *0.2874* |

Data presented as mean ± SD, *n* = 6. Absence of superscript letters on the same row indicates no significant differences between experimental groups (one-way ANOVA followed by Tukey test, *p*<0.05).

The non-enzymatic antioxidant molecule of reduced glutathione (GSH) was approx. 2.84-fold higher in prawns fed the diet supplemented with 3% OG-EO, with the enzymatic antioxidant defence and lipid peroxidation levels constant among all experimental groups (Table 1). Thus, OG-EO may present a non-enzymatic antioxidant bioactivity and contribute to the health and welfare of *M. amazonicum*.

**Table 2.** Antioxidant activity in the hepatopancreas of*Macrobrachium amazonicum* post-larvae fed diets supplemented with different levels of *Ocimum gratissimum* essential oil.

|  |  |
| --- | --- |
| **Antioxidant activity in prawn’s hepatopancreas** | **Experimental groups (g OG-EO kg diet −1)** |
| **0%** | **1%** | **2%** | **3%** | ***p*-value** |
| Lipid peroxidation  | 1.27 ± 0.45 | 1.47 ± 0.70 | 1.41 ± 0.64 | 1.39 ± 0.85 | *0.9147* |
| Reduced glutathione |  3.92 ± 2.33b |  8.75 ± 3.54ab |  9.75 ± 3.41ab | 11.13 ± 4.93a | *0.0134\*\** |
| Catalase | 0.16 ± 0.04 | 0.17 ± 0.05 | 0.21 ± 0.13 | 0.20 ± 0.05 | *0.5460* |
| Glutathione peroxidase | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.01 | *0.1840* |
| Glutathione S-transferase | 0.01 ± 0.00 | 0.02 ± 0.02 | 0.02 ± 0.01 | 0.017 ± 0.01 | *0.3128* |

OG-EG: *Ocimum gratissimum* essential oil. Data presented as mean ± SD, *n* = 8. Different superscript letters on the same row indicate significant differences between experimental groups (one-way ANOVA followed by Tukey test, *p*<0.05).

**Conclusion**

The inclusion of OG-EO in diets can increase the antioxidant status of *M. amazonicum*, particularly the non-enzymatic defence system. Thus, the dietary use of OG-EO as a natural antioxidant is beneficial and can improve the health and welfare of farmed prawns.

**References**

De Souza Valente, C., Coates, C. J., Cagol, L., Bombardelli, R. A., Becker, A. G., Schmidt, D., Heinzmann, B. M., Vaz-dos-Santos, A, M., Baldisserotto, B & Ballester, E. L. C. (2024). Antioxidant status and performance of *Macrobrachium rosenbergii* juveniles fed diets containing non-nutritive *Aloysia triphylla* essential oil. Aquaculture International, 1-14.

Hassaan, M.S., El-Sayed, AMI, Mohammady, EY, Zaki, M.A., Elkhyat, M.M., Jarmołowicz, S., & El-Haroun, E.R. (2021). Eubiotic effect of a dietary potassium diformate (KDF) and probiotic (*Lactobacillus acidophilus*) on growth, hemato -biochemical indices, antioxidant status and intestinal functional topography of cultured Nile tilapia *Oreochromis niloticus* fed diet free fishmeal. Aquaculture, *533*, 736147.