Comparison of mixed Ultrasound waves with the extracts of *Nerium oleander* L. on the growth of cyprid barnacles *Balanus amphitrite* Amphitrite

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Abstract. This study was conducted to demonstrate the effect of plant extracts from the *Nerium oleander* L. plant and ultrasound waves of different frequencies (10-50) (20-45) (50-60) (30-65) KHz on the barnacle larvae cyprid of barnacle *Balanus amphitrite* amphitrite obtained from Nasiriya Thermal Power Station inlet water. Samples were collected bimonthly three replicates. The results of the Oleandrin 1 and 2.5 mg/500 ml showed an effect of losing the larvae cyprid, 2.5 mg showed an increasing effect on the seventh day. A maximum losing of larvae cyprid was attained when using ultrasonic with conjunction of 2.5 mg Oleandrin extract reached 100% on the tenth day.

1. Introduction

Barnacles are marine dominant fouling organisms which accumulate on ship hulls, piers and under water structures. Biofouling by these invertebrates results in an increase in the power and fuel consumption (1). Figure 1 shows a colony of barnacles at water intakes of Nasiriya Province. The growth of barnacles proliferation at port pipes that carry river water to condenser power plants, cause the blockage and closing of those tubes. This will lead to the deterioration in the production of electrical energy in the power plants of Al-Nasiriya power station in the province of Thi-Qar, southern Iraq, leading to losses in the energy production which in most cases exceeds 200MW.

Some technologies were used to deter fouling on stationary objects or niche areas and these include sound (2)(3)(4), vibration (5), Sonication (6), electricity (7)(8), microwave (9) and aeration (10)(11). Moreover, Pulsed electric fields (PEF) on planar-interdigitated electrodes (IDE) have been investigated to deter Pseudomonas aeruginosa biofilms (7). Barnacle cyprids are organisms in aquatic biofouling, they search for a substratum for settlement and metamorphosis (12). As a result of their broad distribution in coastal and estuarine regions (13), and their prominence as a fouling species in warm waters worldwide (14).
The aim of the present paper was to study the effects of ultrasound in inhibition of the growth of cyprid larvae *B. a. amphitrite* and comparison of mixed ultrasound effects with those of extracts of *Nerium oleander*.

2. Experimental Design

2.1 Description of the study area

Nasiriya thermal generation power station in southern Iraq is located on the Euphrates river, and just 7.34 km from the city centre, Which is located "31°2′18.00" North and "46°11′25.66" east of the city centre. The station consists four main units of electric power generation per unit capacity of 210 megawatts/hour, and although of the total capacity of 840 megawatts/hour when the build, but the total production at the present time is between (500-550) megawatt/hour due to, among other thing, growth of barnacles.

The samples were collected from the cooling water processing (C.W.Ps). It is an iron platform containing concrete ponds, down the platform includes a set of pumps needed for water to cool the thermal condensate per unit of electrical energy generation as well as a number of filters (rough, soft, swivel) that operate on the incoming water purification through massive pipes to capacitors. Barnacles settle colonies on the concrete surfaces of the platform as well as the previously mentioned rail parts,
causing clogging of the pipes and block the cooling water taken directly from the river. In addition, some barnacles penetrate through the water pipes to heat exchangers causing the deterioration of their efficiency in thermal exchanger.

![Sample collection site](image)

**Figure 2:** Sample collection site

2.2 Extraction of Oleandrin

Leaves of plant *Nerium oleander* were collected, atmospheric dried samples were ground to fine powder with the aid of a mechanical grinder and sieved through coarse 2.5µm mesh. Then 100g of powder was extracted with 150 ml of methanol to water (90:10) using a homogenizer for 5 minutes. The mixture was then filtered through Whitman No.1 filter paper. The sample was re-extracted following the same above procedure for 4 hour many times. The extracts were collected, and evaporated by rotary evaporator to 10 ml, the extract was stored in a refrigerator until analysis\(^{(15)}\). Ethyl acetate as an intermediate polar solvent to extract Oleandrin through the alcoholic extract after dissolve in water and mixing it with ethyl acetate. A small amount of ammonium chloride NH\(_4\)Cl added to prevent emulsifying phenomenon, then separating funnel left for a period to be separated into two layers. The organic layer was collected, concentrated by a rotary evaporator with controlling the temperature which should not exceed 50 °C\(^{(16)}\).

2.3 Treatment with Oleandrin

The concentrations 0, 1, and 2.5 mg /500 ml of oleandrin extract, were used in three replicates. After the collection river water samples, filtered using a sieve of a diameter of 2.5µm to isolate the Cyprid larvae from the rest of the pond. placed in a container with a volume of 1000 ml. 500 ml of river water was added to the container. Cyprid larvae were monitored during the larvae control period (10 days). The percentage of inhibition was observed daily by utilizing a microscope MT4300L.

2.4 Treatment with ultrasound waves and Oleandrin

To achieve this purpose, the following devices were used: (i) New dual speaker Ultrasound pest Repeller CO-350902 (30-65KHz), (ii) Solar Ultrasound Repeller AN-B030 (10-50KHz). Water
samples were subjected in outlined addition to the extract in 2.3 and specifically with a concentration of 2.5 mg/500 ml for the ultrasonic waves source of frequency (30-65, 10-50KHz) power (2watt). Ultrasound device were located at a distance of 5cm from the container in conjunction with the oleandrin extract, except for the control sample. Cyprid larvae were monitored within 10 days. The percentage of inhibition was observed daily by utilizing a microscope MT4300L.

2.5 Treatment with ultrasound waves and mixed ultrasound with Oleandrin

To perform this purpose, the following devices were used (i) Solar Ultrasound Repeller AN-B030 (10-50KHz), (ii) LW-11DS automatic sweep Ultrasound Repeller YL-35-090200D (20-45KHz). Ultrasound devices were located at a distance of 5cm from the container except for the control sample were subjected to the mixed effect of the extract and Ultrasound. Cyprid larvae were monitored within 10 days of the experiment. The percentage of inhibition was observed daily using a microscope. The following devices were also used to perform the experiment:
(i) New dual speaker Ultrasound pest Repeller CO-350902 (30-65KHz).
(ii) Double wave pest Repeller SS-788 (50-60KHz).
Ultrasound devices were located at a distance of 5 cm from the container in conjunction with the Oleandrin extract (2.5 mg/500 ml) except for the control sample. cyprid larvae were also monitored within 10 days of the experiment.

2.6 Statistical Analysis

Statistical analyses were conducted using social mediated statistical program SPSS (version 19) and using an ANOVA test to find the least significant difference L.S.D below \( p \leq 0.05 \) probabilities of a comparison between the plant extracts and ultrasound and duration of exposure to the treatment. Death percentage of cyprid is corrected according to the Abbott equation knowledge by Schneider and Orell Formula as following as (17):

\[
\text{death percentage of Cyprid} = \frac{\text{% death percentage in the treated sample} - \text{% death percentage in control sample}}{100 - \text{% death percentage in control sample}} \times 100\%
\]

3. Results and Discussion

The collected water samples had cyprid larvae, these larvae are shown in Figure 3

![Figure 3](image-url)

**Figure 3:** Cyprid larva in the samples collected from the Euphrates river in Nasiriya, south of Iraq.
3.1 Impact of treatment with Oleandrin

The results listed in Table 1 and Figure 4 illustrate the effective compound extract of Nerium leaves had an impact in the destruction of the larvae cyprid of barnacle B.a.amphitrite. The results show that there is no significant difference between the working groups appeared in a concentration of 1 mg/500ml compared with the control group during the first day of the study. The highest percentage of the loss of the larvae cyprid was on Day 10. The loss was 12 larva out of 12 larva, and consequently the rate of destruction was 100% compared with the control group in which the loss percentage of 2 larva out of 12 larva and the rate of loss of 16.6% reached.

The results showed that the lowest rate of destruction of the larvae was on first day of the experiment. The loss percentage of cyprid larva was one out of 12 larva and therefore the percentage of loss of 8.3% when using the concentration of 2.5 mg/500ml compared with the control group that did not experience any percentage of the loss of larvae under those circumstances.

The percentage of the loss of larvae cyprid of barnacle B.a.amphitrite increased as the concentration of active compound oleandrin extract increases. Table 1 illustrates that the concentration of 2.5 mg/500ml caused the highest percentage of loss of larvae and the differences are significant compared to the concentration of 1 mg/500ml. Moreover, the table shows that the loss percentage of the larvae spread was 8 larva and the percentage of loss is 59.9% on seventh day.

Table 1: Measurements of Oleandrin impact on the Inhibition on the growth of cyprid barnacles B.a.amphitrite (the total number of larvae = 12).

| Time (day) | Conc.(mg/500ml) | 1     | 2.5   | Control |
|------------|-----------------|-------|-------|---------|
| 1          | 0.00±0.00       | 1.00±0.00 | 0.00±0.00 |
| 2          | 1.00±0.00       | 2.00±1.73 | 0.00±0.00 |
| 3          | 2.00±1.73       | 2.00±1.73 | 0.00±0.00 |
| 4          | 4.00±1.73       | 4.00±3.46 | 0.00±0.00 |
| 5          | 4.00±1.73       | 6.00±0.00 | 0.00±0.00 |
| 6          | 5.00±1.73       | 8.00±1.73 | 1.00±0.00 |
| 7          | 5.00±1.73       | 8.00±1.73 | 2.00±1.73 |
| 8          | 7.00±1.73       | 9.00±0.00 | 2.00±1.73 |
| 9          | 8.00±1.73       | 10.00±1.73 | 2.00±1.73 |
| 10         | 9.00±0.00       | 12.00±0.00 | 2.00±1.73 |
| LSD        |                 |       |       | 3.51    |

* Number of larvae ± Standard deviation
3.2 Effect of mixed ultrasound and Oleandrin

The results listed in Table 2 and Figure 3 illustrate the ultrasound and the extract effective composite plant oleander had an impact in the destruction of larvae cyprid of barnacle \textit{B.a.amphitrite}, the result show that is a significant difference between the working groups appeared the in a concentration of 2.5 mg /500ml compared with the control group during the first day of the study. The highest a percentage of the loss of larvae cyprid was on tenth day. The loss was 12 larva out of 12 larva, and consequently the rate of destruction was 100% compared with the control group.

The results showed that the lowest rate of destruction of the larvae was on Day one of the experiment. The loss percentage of cyprid larva was one out of 12 larva and therefore the percentage of loss of 8.3% when using the concentration of 2.5 mg/500ml compared with the control group that did not experience any percentage of the loss of larvae under those circumstances.

The results showed that the highest rate of destruction of the larvae was on tenth day of the study. The loss percentage of cyprid larva was 12 out of 12 larva and therefore the percentage of loss of 100% when using the concentration of 2.5 mg/500ml and ultrasound waves compared with the control group that did not experience any percentage of the loss of larvae under those circumstances.

This is a unique study of its kind, is considered as the use of ultrasound and the concentration of 2.5 mg / ml of the active compound (Oleandrin) and were compared between the two separately, and in other studies used different frequencies of ultrasound (70 to 445 Hz), the Water basins contain on the larvae. It was observed to increase the vibration amplitude and a decrease in the number of marine barnacles conjoined at the frequency of 260 Hertz \textsuperscript{(18)} \textsuperscript{(19)}.

Table 2: Effect of mixed ultrasound and oleandrin extract on the growth of cyprid barnacles \textit{B.a.amphitrite} (the total number of larvae = 12).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Time (Day) & 1mg & 2.5mg & Control \\
\hline
1 & 3 & 2 & 4 \\
2 & 1 & 1 & 3 \\
3 & 2 & 1 & 2 \\
4 & 1 & 1 & 1 \\
5 & 0 & 0 & 0 \\
6 & 0 & 0 & 0 \\
7 & 0 & 0 & 0 \\
8 & 0 & 0 & 0 \\
9 & 0 & 0 & 0 \\
10 & 0 & 0 & 0 \\
\hline
\end{tabular}
\caption{The effect of mixed time and concentrations of Oleandrin extract on the growth of cyprid barnacles \textit{B.a.amphitrite}.}
\end{table}
| Time (day) | Treatment |  | Control |
|-----------|-----------|---|---------|
|           | Ultrasound waves | Oleandrin extract (2.5mg/500ml) |           |
| 1         | 1.00±0.00     | 0.00±0.00             | 0.00±0.00|
| 2         | 2.00±1.73     | 1.00±0.00             | 0.00±0.00|
| 3         | 3.00±0.00     | 3.00±0.00             | 0.00±0.00|
| 4         | 5.00±1.73     | 5.00±1.73             | 0.00±0.00|
| 5         | 6.00±0.00     | 5.00±1.73             | 0.00±0.00|
| 6         | 6.00±0.00     | 6.00±5.20             | 0.00±0.00|
| 7         | 9.00±0.00     | 8.00±3.46             | 0.00±0.00|
| 8         | 11.00±1.73    | 9.00±3.00             | 0.00±0.00|
| 9         | 11.00±1.73    | 10.00±3.46            | 0.00±0.00|
| 10        | 12.00±0.00    | 12.00±0.00            | 0.00±0.00|
| LSD       |           | 3.46                  |         |

* Number of larvae ± Standard deviation

Figure 3.2: Effect of mixed time, ultrasound and Oleandrin extract on the growth of cyprid larvae of barnacle *B.a.amphitrite*

3.3 Effect of ultrasound waves and mixed ultrasound waves with Oleandrin.
The results listed in Table 3 and Figure 4 illustrate that the ultrasound waves and the mixture (ultrasound + active compound extract) had an impact in the destruction of the larvae cypriid of barnacle B.a.amphitrite. The result also showed that there is no significant difference between the working groups compared with the control group during the first day of the study, as well as, on second day of the working group using a mixture of ultrasound and effective compound, there were no significant differences compared with the control group.

The results showed that the highest rate of destruction of the larvae was on tenth day of the study. The loss percentage of cypriid larva was 12 out of 12 larva, and therefore, the percentage of loss was 100% when using the ultrasound waves and mixed compared with the control group that did not experience any percentage of the loss of larvae under those circumstances. Moreover, the loss percentage of cypriid larva was 12 out of the 12 larva at Day nine of the study.

In this study, the ultrasound waves were used with low frequencies and a mixture of these waves with the oleandrian extractor. As shown in Table 3 when only ultrasound waves were used, the 100% of destruction occurred on tenth day while when the mixture of ultrasound waves and the oleandrian extract were used, the 100% destruction was on Day 9. The use of low frequency (30 Hz) was studied before and there was no destruction of the larvae, but when the waves were applied to the basins full with larvae; it was noted that there was a small and insignificant lack in the number of larvae.

Table 3: Effect of ultrasound waves and mixed ultrasound waves with Oleandrin extract at the same experience on the growth of cypriid barnacles B.a.amphitrite (The total number of larvae = 12).

| Time (day) | Ultrasound waves | Ultrasound waves and Oleandrin extract (2.5mg/500ml) | Control |
|-----------|------------------|---------------------------------------------------|---------|
|           |                  |                                                   |         |
| 1         | 0.00±0.00        | 0.00±0.00                                         | 0.00±0.00|
| 2         | 1.00±0.00        | 0.00±0.00                                         | 0.00±0.00|
| 3         | 2.00±1.73        | 2.00±1.73                                         | 0.00±0.00|
| 4         | 4.00±1.73        | 5.00±1.73                                         | 0.00±0.00|
| 5         | 5.00±1.73        | 6.00±0.00                                         | 0.00±0.00|
| 6         | 6.00±0.00        | 8.00±1.73                                         | 0.00±0.00|
| 7         | 8.00±1.73        | 10.00±1.73                                        | 0.00±0.00|
| 8         | 8.00±1.73        | 11.00±1.73                                        | 0.00±0.00|
| 9         | 10.00±1.73       | 12.00±0.00                                        | 0.00±0.00|
| 10        | 12.00±0.00       | 12.00±0.00                                        | 0.00±0.00|

LSD 2.37

* Number of larvae ± Standard deviation
Figure 3.3: The effect of time, ultrasound waves and a combination of ultrasound waves and extract the effective compound of the oleander plant in the inhibition of the barnacle larvae cyprid B.a.amphitrite.

4. Conclusion
In this study, the impact of using a mixture of extracts of the oleander plant (Oleandrin) and ultrasound waves of different on the cyprid larvae Balanus amphitrite amphitrite which grow on Al-Nasiriya Power Station inlet water pipes was investigated. The rate of losing the larvae cyprid was 100% when using ultrasound with Oleandrin extract of the oleander plant at different frequencies, and this indicates that the ultrasound is a perfect choice with the plant extracts in the inhibition larvae cyprid.

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