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Antifungal activity of turmeric (*Curcuma longa* L.) extract on *Saprolegnia* infected common carp (*Cyprinis carpio* L.) eggs

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Abstract

Common carp [*Cyprinus carpio* L.] eggs in fish hatcheries can be severely infected by water molds, especially *Saprolegnia.* The current study was conducted to investigate the efficacy of alcoholic turmeric [*Curcuma longa* L. extract as a prophylactic measure to prevent infection during the incubation period instead of Malachite Green as an eco-friendly method. Fungi were isolated and identified during the treatment time of the artificial propagation spring season of carp fish 2021; Curcumin was extracted from turmeric [*Curcuma longa* L.] by silica gel column for the removal of essential oil, and its concentration was determined and compared with the standard by HPLC. The percentage of infected eggs, non-infected eggs, hatching eggs, and larvae survival rate after seven days of incubation were estimated. *Saprolegnia* spp. were isolated and identified depending on their morphological features. Deferment concentrations of Curcumin were used [250,500 and 1000] mg\L. There is no significant difference between treatments, but all concentrations are significantly different from Malachite Green. The percentage rate of infected eggs was 0.9±2.2, and non-infected eggs were 98.8±0.4 hatching rate was 55.3± 1.4, and larvae survival rate was 90.4±1.7. Alcoholic extract of Curcumin proved its efficacy as an eco-friendly compound to be successfully used as a prophylactic treatment of carp eggs in the hatchery.

Keywords: Saprolegniasis; Extract of Curcumin; Common carp; Eggs hatchery

1. Introduction

Aquatic environments are a good media for several biological communities, including aquatic fungi. Water molds have environmental importance, especially those belonging to the order Saprolegniales, which infect various types of fish and their fingerlings, eggs, and adults. The disease, called saprolegniasis, is considered the most important harmful factor in fish hatcheries of freshwater fish and their eggs [1]. In fish hatcheries, Saprolegniasis usually controlled with Malachite Green [2]. Due to its carcinogenic characteristics, its use is restricted in addition to its side effects [3,4]. Fish eggs' cellular structure, like amino acids Aspartic acid, Glutamic acid, and carbohydrates, act as attractive factors that stimulate the direct movement of the Zoospors toward fish eggs during the incubation period [5]. Previous studies discussed using plant extracts as an eco-friendly alternative method to control diseases and animal health [6,7,8]. It was recommended to be safe for fish and applicable for fish hatcheries owing to their beneficial effects in enhancing fish resistance. *Aeromonas hydrophila* [9]. The inhibitory effect of plant extract on *Saprolegnia parasitica* growth was examined in vitro conditions, and its anti fungal property was applied by using the extract in treatment water [10,11]. Curcumin's antibacterial, anti fungal, and toxic activities on *Saprolegnia parasitica* were confirmed [12]. *Saprolignia* disease is controlled in carp egg hatcheries with a therapeutic bath of alcoholic Curcumin extract, which has been shown to be as effective as a substitute for malachite green.

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2. Material and methods

2.1. Preparation turmeric extract

Dried turmeric [*Curcuma longa* L.] was ground into powder samples of 10 grams; turmeric was impregnated with silica gel (16 g), and the sample was loaded onto a silica gel column 200 grams and diluted in1000 ml of hexane. To get 400 ml fractions, the column was diluted with increasing polarity of benzene and ethyl acetate. A mixture of benzene70ml and Acetone 30 ml was used to elute the compound [13].

2.2. High-performance liquid chromatography assay for Curcumin

Methanol stock solutions of Curcumin were prepared at a concentration of 0.5 mg/ml according to [13] method. The flow rate was 1.0 ml/min at ambient temperature during elution with gradient solvent systems. There were three mobile phases: methanol (I), 2% acetic acid (II), and acetonic (III). A linear program of acetonic solvents in (II) was used to determine quantitative levels of Curcumin for the solvents above over a period of 0-15 min. The gradient then went from 65 to 45% acetonic in B for 15-20 min, with a constant of 5% (I). Ten micro liters of each of the working standard solutions 2.0 µg of standard Curcumin, was injected into the HPLC. All solvents/chemicals used were of HPLC grade obtained from (Sigma).

This research was achieved during the spring breeding season of 2021 for four days for egg incubation and three days after hatching in the Al-Wahda fish hatchery south of Baghdad. After Carp eggs fertilization, desired number (5000 ±10) of eggs per liter was obtained, and fertilized eggs were cultured in (Zoug Jars) Switzerland system (Figure 1 and Figure 2). Physiological and environmental factors include pH, temperature, and water salinity daily measured and monitored.

2.3. Treatment included five groups of Carp fertilized eggs

- negative control group without the use of any disinfectant,
- Positive control with a malachite green concentration of 0.5 mg/l for 5 minutes was done once daily and applied as indicated by [14].
- Alcoholic extracts of Curcumin with the following concentrations: 250, 500, and 1000 mg/l twice per day [15].

Each treatment yielded 5000 ±10 eggs /L. After 24 hours of incubation, eggs were randomly sampled to estimate the percentage of fertilization depending on [16]:

$$\text{Percentage fertilization \%} = \text{total count of fertilized eggs} / \text{total count of eggs} \times 100$$

$$\text{Hatching rate \%} = \text{count of hatching fries} / \text{amount of fertilized eggs} \times 100$$

Disinfecting treatment was achieved by sinking the incubated eggs in the bath for 5 min with use concentrations of 250, 500,1000 mg/L of the alcoholic extract of Curcumin twice daily; the eggs were siphoned from the jar into the pan, and air currents were blown through it was set up inside the jar. The larvae of hatchery fish are transferred from jar incubators to trucks after four days. They are separated from their eggs as they will suffocate if they are exposed to too much oxygen. Disinfection treatment of larvae continued in pans and aeration flow was performed. In each treatment, after the eggs had hatched all the way, the number of fungal-infected eggs was calculated as follows (17):

$$\text{Fungal -infected eggs rate \%} = \text{count of infected eggs} / \text{total amount of eggs} \times 100$$

A separate tray of larvae for each treatment was used to monitor if there was any change in body shape and determine how each treatment affected larvae appearance for a week after hatching.

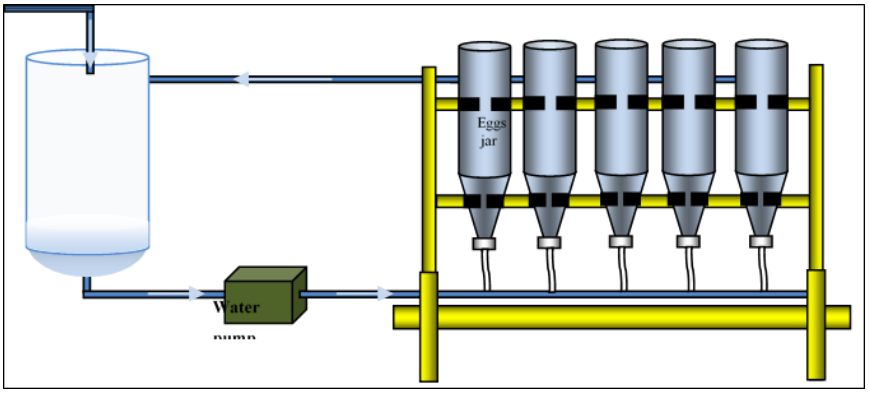

Figure 1 Illustration figure Closed circulatory incubation system for fish eggs incubation

Figure 2 Closed circulatory incubation system for fish eggs incubation (Zoug jar) Switzerland system in Al-Wahda fish hatchery

2.4. Statistical analysis

For each treatment, a calculation was made to the mean and standard deviation (SD) using Excel (Microsoft). The D'Agostino-Pearson tests were used to evaluate the normality of data arrangement; differences were considered to be statistically significant at (p-value < 0.05).

3. Results

3.1. Curcumin determination by HPLC

Curcumin was isolated from turmeric (*Curcuma longa* L.) by silica gel column after initial elution with hexane for the removal of essential oil. Separation was achieved on elution with alcohol mixtures with increasing polarity. HPLC analysis of standard Curcumin one peak at (6.12 mins) retention times and 6.21 min for alcoholic extract (Figure 3).

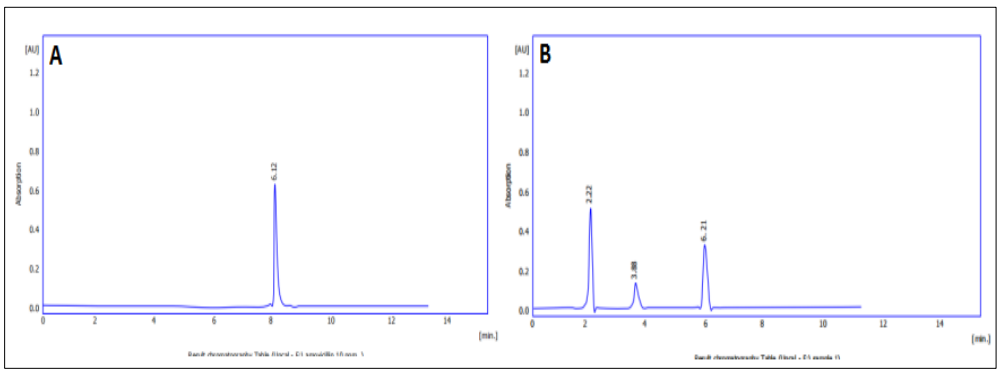

Figure 3 HPLC chromatography of Curcumin: [A] Standard Curcumin [B] Alcoholic extracted Curcumin

3.2. Assessment of larvae Survival Rate % of Hatched carp eggs

During the spring artificial breeding season of 2021, based on statistical analysis, the fertilization rate % and survival rate of embryos correlated significantly p-value < 0.05. Similarly, a positive correlation existed between the fertilization rate and the survival rate of larvae, while a positive and non-significant relationship existed between the survival rate of embryos and the survival rate of larvae.

3.3. Anti fungal effect of Alcoholic Extract of Curcumin

Fungal infection recognized as cotton like growth surrounding dead eggs (Figure 4 c) in all incubators and identified as Saprolegnia spp. Depending on the morphological features(18). Based on the extract concentration, the infection percentage for fish eggs varied. When eggs were treated with 250 mg/L concentration of curcumin extract, the percentage of non-infected eggs was 91.1±0.4. Statistical analysis evidenced that treatment with less concentration of extracts was significantly less than positive control and other concentrations, as shown in (Table 1).

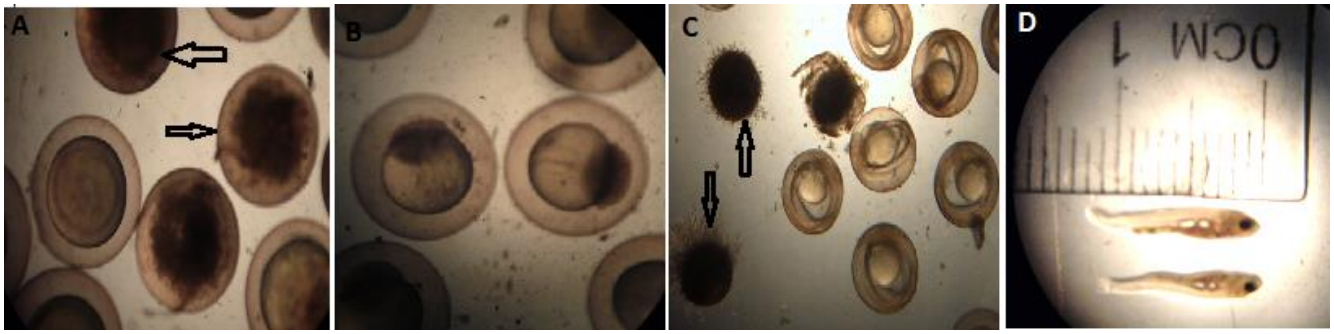

Figure 4 Carp eggs development [A] fertile and rows non-fertile eggs, [B]: eggs in Morula stage, [C] eggs after 48 hours of incubation rows infected dead eggs, [D] Carp larvae after seven days of hatching. Magnification power 10 X

Table 1 Anti fungal effect of alcoholic extract of Curcumin in eggs with different concentrations during incubation period four days results represented by Mean± Standard Division

Treatment	Negative Control	Positive control MG 0.1 mg/L	Alcoholic curcumin extract		
			250 mg/L	500mg/L	1000 mg/L
Eggs fertility rate	82.22±3.8a	82.4±3.4a	82.3±1.2 a	82.8±1.9a	82.9±0.2a
Percentage of infected eggs	72.3±0.6a	2.3±1.9b	2.3±1.7b	1.1±0.1b	0.9±2.2b
Percentage of non-infected eggs	12.7±1.5 c	90.3±0.9b	91.1±0.4a	96.4±0.9a	98.8±0.4a
Percentage of hatching eggs	9.5± 1.2b	46.7± 1.4a	54.3± 2.3ab	49.6± 2.1a	55.3± 1.4a
Larvae survival rate in 7th day [%]	8.3±1.2 c	80.4±4 b	90.5±2.2 a	91.5±2.8a	90.4±1.7a

Different alphabetic letters within the row represent significant differences between treatments at (p-value < 0.05).

All concentrations of alcoholic curcumin extract recorded high levels of non-infected eggs (98.8±0.4) were obtained, followed by (96.4±0.9) and (91.1±0.4). There were no significant differences between them, but all these treatments were significantly different from the positive control (treatment with Malachite Green). At the same time, the lower level was negative control (12.7±1.5), as shown in (Table 1). The Percentage of infected eggs recorded the highest value in negative treatment (72.3±0.6) which was significantly different from other treatments. The survival rates of produced larvae were significantly higher in all curcumin extract concentrations than in the control treatment.

4. Discussion

According to the results, all the examined alcoholic extracts of Curcumin inhibited fungal infection. It has been found that medicinal plants have antiviral, antibacterial, and anti parasitic properties [19]. Curcumin has antioxidant and antimicrobial properties, so it can be used as an alternative to antibiotics to prevent infectious diseases and promote growth [20]. Efficacious inhibitory substances have been detected by HPLC assay; the major active compound was Curcumin in turmeric extract, as noticed by HPLC. According to [21], *Curcuma aromatica* alcoholic and aqueous extracts

treated at doses of 200 mg and 400 mg produced similar results. Our results were in good agreement with previous studies; in demonstrating the inhibitory effects of Tumeric on more than 20 species of fungi, such as *Aspergillus flavus* and *Fusarium verticillioides* [22]. Curcomin inhibits fungal growth by disrupting plasma membrane integrity and mitochondrial dysfunction, leading to metabolic stagnation when used against *Aspergillus flavus* [21,22]. During artificial propagation, fish eggs are exposed to fungal infections, particularly Saprolegniaceae genera, which are common in hatcheries and cause large losses [23].

Using 1000 mg/L of Curcumin alcoholic extract produced the highest prophylactic treatment concentration, which has a greater effect on fungi than the lowest concentrations [24]. Concentrations of 250,500 and 1000 mg/L were significantly higher than Malachite Green. The high concentration of the active compound was in the extract with a concentration of 1000 mg/L [25]. Earlier studies showed that high concentrations of plant extracts cause fungi colonies to shrink due to their obvious effect [26]. The inhibitory effects of the material decrease with lower concentrations as the active compound disperse away from fungal cells, or the low concentration makes the compound of non-therapeutic benefit. The diluted form of some compounds may penetrate better and be more effective in the cells, while the high-concentration form may become concentrated and more complex[27].

There was a variation in the number of larvae produced for each treatment. The reason may not always be due to a particular substance being used during treatment; there was a strong correlation between the number of larvae produced and the mothers' feed, primarily amino acids, vitamins, carbohydrates, and minerals. In addition to producing large-size eggs with high nutritional content, these priorities are essential for ovulation [28]. Many anti fungal agents are used in hatcheries to prevent the spread of Saprolegniasis infections. Plant extracts containing active substances can be considered natural substances; it is possible for some of these compounds to cause side effects in fish, the environment, or consumers during or after treatment. Some compounds can lead to fish loss or may affect important organs of the fish's body, such as the gills and digestive system [29]. The results showed that no distorted larvae were obtained. However, the percentage rate was good as compared with the control. The cause of deformation may be due to the use of a random inbreeding culture. Fishes may have an undesirable appearance because of the emergence of transgenic attributes on recessive genes and then the emergence of an offspring with mutilated limbs, as occurs in the local breeding herds; fish eggs are routinely treated with malachite green, which may lead to this problem [29].

5. Conclusion

The alcoholic extract of Curcumin proved its efficacy as a safe compound to be applied as a prophylactic treatment of Carp eggs in the hatchery. A significant advantage of Curcumin is its high effectiveness as a substitute for malachite green; furthermore, common carp larvae produced did not suffer from malformation and reached a survival rate of 91%.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no competing interest.

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