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Effect of herbal extracts on pathogen *Vibrio harveyi* of *Penaeus monodon*

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Abstract
The present research work is arrived to nullify pathogenicity of *V. harveyi* on *P. monodon* through medicinal plants, a healthy, eco-friendly traditional method. The pathogenicity of *V. harveyi* causes entirely loss in the shrimp culture farms and the use of commercial anti-biotics for disease control causes are highly toxic, side effects to the environment and consumers. Hence the herbal extracts of Neem leaf and Turmeric rhizome were tested to nullify the effect of pathogen, along with the commercial antibiotics like ciprofloxacin, Amoxicillin, Tetracycline and Ampicillin to compare the effect on *V. harveyi*. The methanolic extracts of plants and antibiotics were examined through well diffusion method and MIC also studied. Curcuma and ciprofloxacin exhibited high anti vibrio activity. The study revealed that the rhizome extract of curcuma is the best herbal product for reducing the effect of opportunistic pathogen *V. harveyi* which is abundant in gastro-intestinal tract of shrimp and causing a great loss to the aqua farms.

Keywords: *Vibrio harveyi*, neem, curcuma, antibiotics, *P. monodon*

1. Introduction
In present days, shrimp farming is the most essential feed productivity sector in the world [1]. Many vibrio species cause heavy mortality and they are main problem in cultivating shrimps in Asia [2]. Among the vibrio species, *Vibrio harveyi* pathogen cause infections and entire mortality in hatcheries and cultivation ponds [3-6]. The commercial antibiotics used to eliminate bacterial diseases, but they can cause adverse side effects, such as poisoning of bacteria, accumulation of antibiotic residues in the tissue of shrimps and toxic antibiotics delivered into surroundings. Antibiotic toxins cause many problems to animals and human beings [7]. There is a growing concern about antibiotic resistance in harmful Vibrio strains, like *Vibrio harveyi* [8]. This resistance makes it harder to fight shrimp diseases and there is a need for effective, safer alternatives [9]. Due to this reason, the herbal products are alternatives to antibiotic usage in shrimp farms. The potential use of certain herbal extracts for controlling shrimp diseases have been evaluated [10-11]. Neem (*Azadirachta indica*) and turmeric (*Curcuma longa*) have a long history of use in traditional medicine for various ailments [12]. Neem leaves, seeds, and roots have known antibacterial and antifungal properties [13, 14], effective against *Staphylo coccus, Streptococcus, and Pseudomonas* species [15, 16]. Similarly, turmeric is widely used in Indian medicine for its antibacterial, antifungal, and anti-inflammatory properties [17-20]. This study investigates whether methanolic extracts of Turmeric and Neem can be effective substitutes for antibiotics in fighting *Vibrio harveyi*, a shrimp pathogen.

2. Material and Methods
2.1 Medicinal Plants Collection
Fresh, healthy plants like *Azadirachta indica* (Neem) leaves and rhizome of *Curcuma longa* (Turmeric) were gathered and cleaned with distill water to take off dust particles from the plant material.

2.2 Preparation of plant extracts
Leaves and rhizomes were shade dried and make a fine powder for plant extraction, 100 mg of powders were rinsed with 250 ml of methanol for couple of days [21].

The sludge was then filtered and centrifuged at 20,000 g for 30 min. The solvent residues of the extracts were evaporated at 35 °C. Remaining plant extracts were used to perform the present research work.

2.3 Anti-Vibrio Assay

V. harveyi strain brought from Chandigarh (MTCC No- 3438) and used as the pathogenic bacteria for anti-vibrio assay. *In-vitro* anti-vibrio activity was screened by utilize TCBS agar (TCBS). Petri plates were prepared by pouring 15 ml of sterilized TCBS medium. After solidification (5 minutes), 20 µl of bacterial inoculum was uniformly swabbed and dried (5 minutes). Plant extracts (500 mg) were dissolved in DMSO and different concentrations (100, 75, 50, 25 µg) were prepared from the stock solution. These were then applied using the Agar well diffusion method [22]. Simultaneously, Standard antibiotics (Ciprofloxacin, Tetracycline, Amoxicillin, Ampicillin) were used as controls. Different concentrations (10, 1, 0.01, 0.1 µg) were prepared and applied using the Disc diffusion method [23]. Plates were incubated at 35 °C for 24 hours. Zones of inhibition were observed, and their diameters were measured in millimeters. All experiments were performed in triplicate.

2.4 Minimum Inhibitory Concentration (MIC)

The Neem and Curcuma extracts exhibiting anti-Vibrio activity in the agar well assay were further evaluated for MIC using a serial dilution method [24]. The extracts were diluted to various concentrations ranging from 2 to 30 mg/ml. The MIC was determined as the lowest concentration showing a clear zone of inhibition. This experiment was also Carryout in triplicate.

2.5 Statistical Analysis

Results are represented as mean ± standard error of the mean (SEM). To assess the significance of differences, a t-test was used to compare the control group with treatment groups, while a one-way analysis of variance (ANOVA) was employed to compare the control group with various treatment means. A p-value less than 0.05 was considered statistically significant.

3. Results

Fig 1: Antibacterial sensitivity of different plant extracts against shrimp pathogen *Vibrio harveyi.*

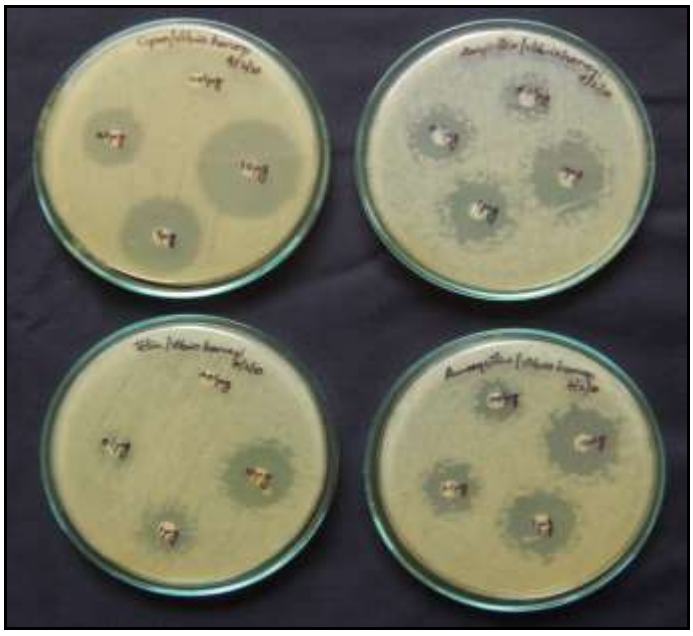

Fig 2: Antibacterial sensitivity of different antibiotics against shrimp pathogen *Vibrio harveyi.*

Figure-1 shows the inhibition zone of *Vibrio harveyi* with Turmeric and Neem extracts. The highest anti-vibrio activity was found in methanolic extract of curcuma 100 µg (12.4±0.2 mm), 75 µg (10.1±0.1 mm), 50 µg (7.1±1.1 mm), and 25 µg (5.1±1.0 mm), followed by methanolic extracts of Neem 100 µg (10.1±0.1 mm), 75 µg (6.9.±1.1 mm), 50 µg (6.1±1.1 mm), and 25 µg (3.1±1.0 mm) respectively. The methanol extract of Turmeric was the most effective than Neem. Similarly figure-2 shows the inhibition zone of *Vibrio harveyi* with different types of antibiotics. Ciprofloxacine shows highest inhibition zone compare to other antibiotics. The MIC value of Turmeric and Neem extracts were also investigated using Well diffusion method. The MIC value of methanolic extracts of Turmeric and Neem extract with *V. harveyi* was 14.63 and 11.47 ppt, respectively.

Table 1: Antibacterial activity of Turmeric and Neem extracts on *V. harveyi* at different concentrations.

Herbal plants	Zone of Inhibition (mm) Concentration of plant extracts in (µg)			
	100	75	50	25
Turmeric	12.4±0.2	10.1±0.1	7.1±1.1	5.1±1.0
Neem	10.1±0.1	6.9.±1.1	5.1±0.2	3.1±1.0

(mean ± SD mm; n = 3)

Table 2: Antibacterial activity of different antibiotics on *V. harveyi* at different concentrations.

Antibiotics	Zone of Inhibition (mm) Concentration of antibiotics in (µg)			
	10	1	0.1	0.01
Ciprofloxacine	17.4±0.3	12.4±0.1	9.4±0.1	--
Tetracycline	11.2±0.1	8.5±0.2	--	--
Ampicillin	14.9±0.2	10.9±0.1	8.9±0.2	3.1±0.2
Amoxyllin	15.5±0.2	13.6±0.0	10.4±0.1	6.6±0.1

(mean ± SD mm; n = 3). --- indicates no Inhibition zone.

4. Discussion

The goal of the current research work is to investigate the ability of two medicinal plants Neem and Turmeric extracts to inhibit the growth of shrimp pathogen *Vibrio harveyi.* This is possible alternatives to common antibiotics in shrimp farms. In Accordance to [25], treating bacterial infections in shrimp involves using larger quantities of antibiotics in the culture medium. A large quantity of antibiotics causes many serious problems to animals and human health. An alternative methods are required to reduce antibiotics usage and enhance utilization of herbal products, They have antimicrobial properties instead of commercial antibiotics.

In the current study represents, methonolic extract of Neem leaves and Turmeric rhizome were used to determine their anti-vibrio activity against shrimp pathogen *Vibrio harveyi.* The findings showed that both Neem and Turmeric extracts at a concentration of 100 µg were effective against *Vibrio harveyi* and shows slight to moderate zone of inhibition. Neem leaves contain various antimicrobial components, including alkaloids, glycosides, flavonoids, phenolics, steroids, triterpenoids, carotenoids, and azadirachtin (a potent compound). Previous research [26-33] supports the antibacterial properties of turmeric rhizome extracts against various bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium.* Curcumin, a key component of turmeric, has also been shown to be effective against Helicobacter pylori, *P. aeruginosa*, and *Klebsiella pneumonia.* It is also noticed that the immune system of shrimp got stimulated by medicinal plants. It's important to note that the inhibition zones produced by Neem and Turmeric extracts were smaller compared to those of commercial antibiotics. This suggests that the active potent compound in these herbal extracts might be less concentrated than those in antibiotics. Pathogenic bacteria displayed the highest sensitivity towards antibiotics, as evidenced by the largest inhibition zone. This aligns with findings from another study [34], where 97% of *Vibrio harveyi* isolates exhibited high sensitivity to antibiotics. The shrimp pathogen *Vibrio harveyi* an main infectious bacteria in shrimp farms. The current result revealed that both plant extracts were efficient for suppressing the growth of *V. harveyi* mostly due to its bactericidal effect.

5. Conclusion

Azadirachta indica (Neem) and *Curcuma longa* (Turmeric) plants which have lot of medicinal properties, because of this reason they are used to treat many bacterial diseases and possess the antibacterial activities. Commercial antibiotics shows resistance to bacterial diseases and they cause many side effects to animals and human beings. For this reason, we need alternative methods instead of commercial antibiotics utilization. Neem and Turmeric plants are essential for better, attentive, eco-friendly and cheapest drugs can be developed for curing various shrimp diseases, infections and also they improve shrimp immune system.

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