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Shelf life Study of Freshwater Fishes Using Neem (*Azadirachta indica*) Extract as a Natural Preservative

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A B S T R A C T

This study was performed with aim to replace chemical preservatives used for fish preservation. An experiment was conducted to enhance the shelf life of collected fishes by using herbal treatment. In this research, Neem (*Azadirachta indica*) extract of leaves was used as natural preservative for fish to preserve the freshly captured fish. Dried powder was used for preparing aqueous extract. Microbial loads were estimated by using total plate count. The aim of using this method was to observe bacterial growth between the unpreserved fishes and the fishes treated with preservative. The present study revealed that the use of neem, in the forms of dried leaves extracts shows the inhibitory activity against spoilage causing bacteria and enhances the shelf life of fresh water fishes. This showed a good alternative to enhance the shelf life and quality of fishes at ambient temperature in the areas where no refrigerated systems are available for fishermen.

Introduction

Fishes are important food components from ancient time (Noor *et al.*, 2013) and it is highly perishable food. The preservation of fishes is difficult due to its high perishability (Musa *et al.*, 2010; Okoro *et al.*, 2010 and Dewi *et al.*, 2011). Microbial growth and metabolic degradation are major causes of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Emborg *et al.*, 2005; Dalgaard *et al.*, 2006). A study reported that 30% of landed fishes are spoiled through microbial activity (Amos, 2007). So, there is need of different preservation techniques to

prevent fish spoilage and to enhance the shelf life. These techniques are designed to inhibit the microbial spoilage and the metabolic changes that result in the loss of fish quality.

In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of food preservation that minimize side effect to humans and environment, newer biological preservatives are developed to replace deleterious chemical preservatives. Neem (*Azadirachta indica*) is a traditional and highly esteemed medicinal tree in Indian sub-continent. Biological activities and medicinal properties of neem have been extensively reviewed by Biswas *et al.,* (2002). One of the most promising natural compounds is azadirachtin, an active ingredient extracted from the neem tree, whose antiviral, antibacterial and antifungal properties have been known for several years (Isman *et al.,* 1990; ICAR, 1993 and Harikrishnan *et al.,* 2003). Therefore, neem-based preservatives are being investigated as alternatives to synthetic chemical preservatives for the preservation of food. In this study an attempt has been made to extend the shelf life of newly caught fishes using aqueous extract of dried neem leaves.

Materials and Methods

The material like Neem leaves were collected from neem trees (*Azadirachta indica*) of MLSU Campus, Udaipur. The fresh fishes were collected from Fatehsagar Lake, Udaipur (Raj.) with the help of local fisherman for the study.

Preparation of aqueous neem leaf extract

The extract of green sun dried leaves of neem*)* was used as natural preservative for fish. Neem leaves were collected and washed twice with distilled water. Then the leaves were sun dried and mechanically powdered. Dried powder was used for preparing crude aqueous extract. Dried powdered material was suspended in distilled water for 24 hours. Then, the mixture was filtered with buckner funnel using vacuum pump and filtrate was used for experiment. The concentration of aqueous extract was 2.0 X 10^5 ppm.

Treatment of experimental fishes

Fresh fishes were collected with the help of local fisherman and brought to laboratory under hygienic condition. All the fishes were divided in 2 sets i.e. one set as control and another as experimental fish. For the dipping experiment, one set of the fish samples were dipped into preservative solution (aqueous extract of dried leaves of neem). Another set of fishes were also put in the empty sterile container as control. Following the dipping process, the both sets of fishes were allowed to stand at room temperature for 24 hours.

Bacterial load estimation

The total bacterial count for the fishes is determined using the plate count agar (Hi Media, Mumbai, India) after incubating the plates for 48 hours at 37^0C in appropriate dilutions (APHA, 1994). Microbial loads were estimated for 24 hours at every 2 hours interval. The 0 hour (*i.e.* no delay) fish samples were kept for the growth of bacteria. The aim of using this method was to observe bacterial growth between the unpreserved fishes and the fishes treated with preservative.

Results and Discussion

The bacterial load was determined at every 2 hours interval for 24 hours storage of fishes at ambient temperature (Table-1). The bacterial loads at 0 hour were determined for both the experimental sets. Initially (0 hour) the bacterial load found on control fishes

(untreated) was 34 x 10^4 cfu/gm and on treated fishes it was 41 x 10^4 cfu/gm. The initial bacterial loads for both sets were found in same range i.e. 10^4 cfu/gm. This range was constant at 6 hours in treated fishes, while in untreated fishes it reached to 17 x 10^5 cfu/gm in 4 hours.

After 8 hours, bacterial load in non treated fishes reached upto 10^6 cfu/gm (19 x 10^6 cfu/gm). While at the same time (after 8 hours) in treated fishes the bacterial load was 10^5 cfu/gm (12 x 10^5 cfu/gm). A study indicates that when the bacterial load reached upto 10^6 cfu/gm at this level the fish was found to be organoleptically unacceptable for consumption and it is considered that these food items are spoiled (Shewan, 1970). It means after 8 hours nontreated fishes were spoiled and not safe for human consumption.

After 16 hours treated fishes showed the total bacterial load 10^6 cfu/gm (27 x 10^6 cfu/gm), which was not under the acceptable limit. The results further showed that after 16 hours the treated fishes were organoleptically spoiled. In comparison of non treated fishes (as control) and treated fishes, it was observed that non treated fishes were spoiled in 8 hours, while treated fishes were spoiled in 16 hours. It was concluded that the microbial loads were suppressed in treated fishes and the spoilage time duration was enhanced, it means the shelf life of freshwater fishes increased by two folds using this treatment. In treated fishes, it was observed that the total bacterial loads were increased slowly.The overall bacterial growth rate also decreased but in treated fishes during the initial 16 hours, bacteria did not grow with exponential growth but after 16 hours, they showed exponential growth (Figure 1).

Growth rate of bacteria was measured to understand the effect of neem extract on bacterial load of fish. The bacterial load results were converted in $\log_{10}$ base value and growth rate was calculated. The growth rate was calculated between 0-8 hours, 8-16 hours, 16-24 hours and the overall growth rate was calculated with 0-24 hours (Table- 2). The growth rate of control group (un-treated) and experimental group (treated with aqueous extract of neem) was compared with 0-8 hours, 8-16 hours and 16-24 hours. Initially for control group the growth rate was 3.49% whereas it was only 1% for experimental group in time interval of 0-8 hours. It showed the effect of neem extract. Within 8-16 hours it was 2.64% for control fish and 2.54% for experimental fish. Within 16-24 hours it was 2.32% for control fish and 3.27% for experimental fish, hence it can be concluded that in the initial 8 hours the growth rate was high for control fish whereas it was very low for experimental fish due to the treatment with neem extract. But when storage time increased the growth rate between 8-16 hours almost same for both the groups i.e. control (2.64%) and for experimental (2.54%). The growth rate between 16-24 hours for control group was reduced slightly (2.32%) but for experimental group it was increased to 3.27%. The data shows that the effect of neem extract decreased with increase in storage or holding time. A study indicates that plant based pesticides contain active molecules with low half-life period as compared to chemical pesticide (Sharma *et al.,* 1995). From this point of view it was concluded that after 16 hours the chemical composition of neem extract started degrading. The growth rates for 0-8, 0-16 and 0-24 hours were compared and it was found that for control group the growth rate of bacterial load showed decreasing trend (3.49% - 3.07% - 2.82%) whereas for experimental group it showed increasing trend (1.00% - 1.77% - 2.27%). The growth rate between the control (untreated) and experimental (aqueous extract of Neem) with regular intervals (0-8 hrs, 0-16 hrs, 0-24 hrs, 8-16 hrs and 16-24 hrs) are indicated in

graphics (Figure- 2). The decline of fish from natural aquatic resources and increasing demands for fish, shrimp and other aquatic organisms by consumers are the two main factors for the expansion of aquaculture nowadays (Dogruez *et al.*, 2008). Microbial condition of live fresh fish is normally considered to be sterile. However, microorganisms normally find on the skin, in the gills and also naturally in the guts. Normally the numbers of microorganisms found on the skin of fishes are in range of 10^2-10^7cfu (colony forming units)/cm^2 and in the gills and intestine in range of 10^3-10^9cfu/g (Liston, 1980).

Table.1 Comparative results of treated fishes (aqueous extract of sun dried leaves of neem) and untreated fishes with time duration

S. No.	Fish at ambient temperature without any treatment (Control)		Fish treated with aqueous extract of sun dried leaves of neem and kept at ambient temperature (Experimental)	
	Holding time (hours)	Bacterial Load (cfu/gm)	Holding time (hours)	Bacterial Load (cfu/gm)
1	0	34×10^4	0	41×10^4
2	2	91×10^4	2	56×10^4
3	4	17×10^5	4	68×10^4
4	6	70×10^5	6	86×10^4
5	8	19×10^6	8	12×10^5
6	10	57×10^6	10	26×10^5
7	12	12×10^7	12	47×10^5
8	14	73×10^7	14	76×10^5
9	16	93×10^7	16	27×10^6
10	18	24×10^8	18	74×10^6
11	20	17×10^9	20	38×10^7
12	22	47×10^9	22	81×10^7
13	24	60×10^9	24	41×10^8

Table.2 Analysis of bacterial growth rate with time interval

Time Interval	Without Treatment	Treated
0 - 8 hrs	3.49%	1.00%
8 - 16 hrs	2.64%	2.54%
16 - 24 hrs	2.32%	3.27%
0 - 16 hrs	3.07%	1.77%
0 - 24 hrs	2.82%	2.27%

Figure.1 Bacterial load with time duration between the control (non treated) and experimental (treated with neem extract) fishes

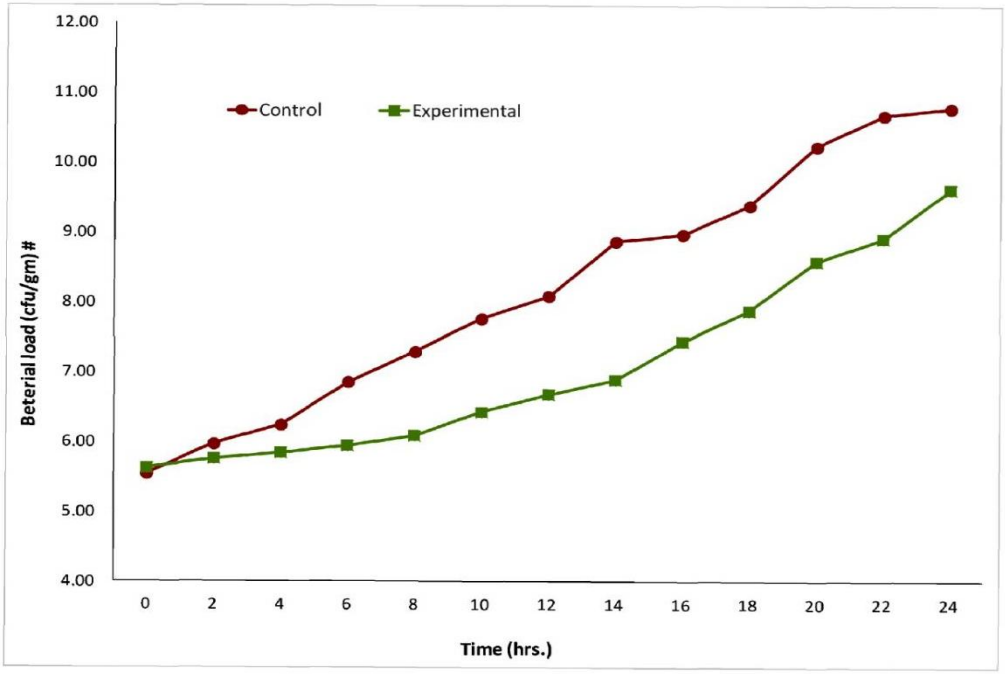
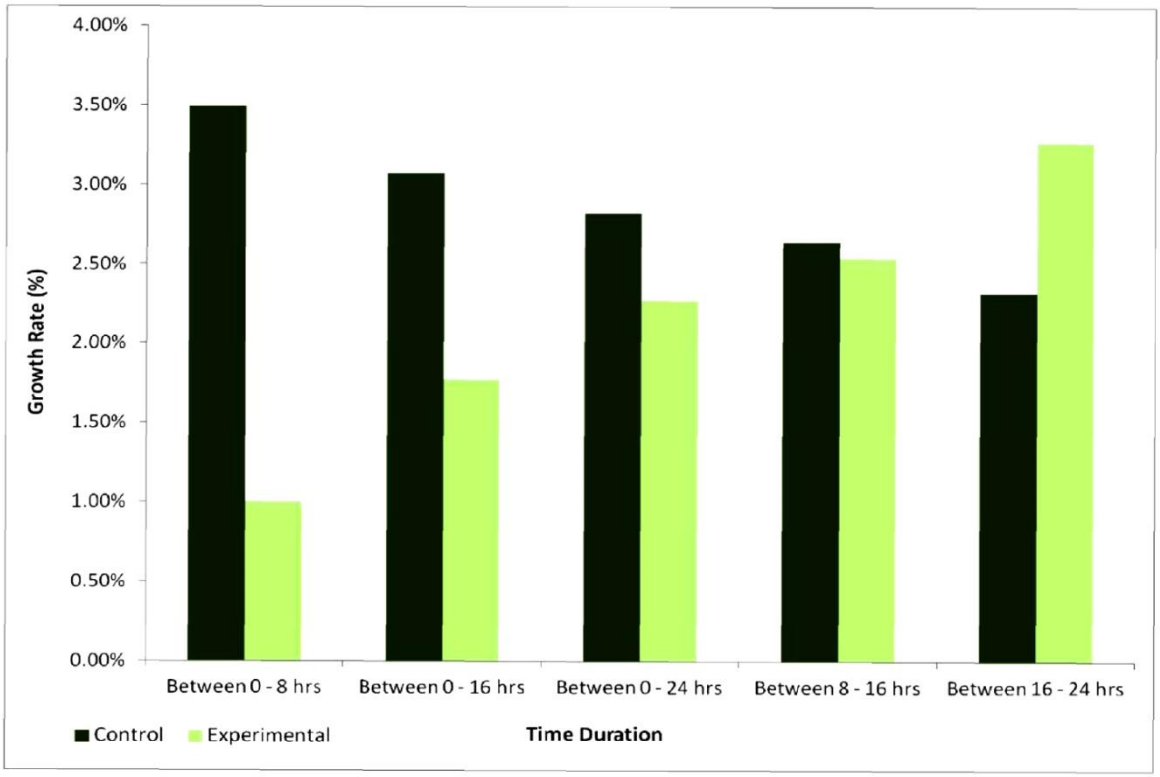


Figure.2 Growth rate with time interval between the control (non- treated) and experimental fishes (treated with neem extract)



Neem has also been used successfully in aquaculture systems to control fish predators (Dunkel and Ricilards, 1998). Martinez (2002) stated that aqueous extract of neem leaves and other neem-based products have been extensively used in fish-farms as alternative for the control of fish parasites and fish fry predators such as dragon-fly nymphs.

Fan *et al.,* (2008) reported that, Tea Polyphenols (TP) dip treatment on the fish samples was to enable the good quality characteristics to be retained for longer period and to extend the shelf life during the iced storage. Similarly in the present investigation, it was observed that aqueous extract of neem leaves can extend the shelf life of fish, which is in concurrence with the work of Fan *et al.,* (2008). The extracts of instant green tea and black tea have been found capable to suppress the bacterial growth in the fish samples (Nugraha *et al.,* 2012) which is again in agreement to the findings of the present research. In support of present study Mousa *et al.,* (2008), stated that the application of neem leaf extract can be used to control unwanted organisms in ponds as environment friendly material instead of deleterious pesticides. Further, the outcome of present research also stresses the need for conducting similar research efforts specially to address issues like sensory analysis with respect to the senses of sight, smell, taste, touch and hearing.

Conclusion of the study is as follows:

To use total fish production optimally is affected by many factors; like lack of facilities, unavailability of ice in remote area, and lack of knowledge of fish preservation particularly among traditional fishermen. There is still a problem of facilities such as good roads for transportation and nearby fish market equipped with cold storage. Still, the best way to preserve fresh fish is by using ice in a ratio of 1:1 (Wibowo and Yunizal, 1998). However, all the fishermen cannot purchase the ice because of economic problem or timely availability. Lack of knowledge and economic problems have led them to use dangerous chemicals as food preservatives. Thus, an alternative of ice and chemicals are needed for these fishermen particularly those who live in remote coastal regions. However, proper food preservation is now a necessity. In addition, many foods that are traded currently travel long distances, often from country to country, having to be preserved one way or another before reaching to the table in the best possible conditions. Preserving food is a way to make food wins "life" to come to our table. While there is nothing like fresh food, there are many ways to conserve natural food in a healthy way, without so many chemical additives. The results of this study justified that dried leaves extracts from neem may be good alternative of other preservatives. The aqueous extract of neem was capable in suppressing bacterial growth in fishes and can also enhance the shelf life of fishes.

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