Antibacterial effect of the optimum anesthetic dose of thyme oil, AQUI-S, clove oil and quinaldine

Amani Al-Yaqout, Azad Ismail, Mohammed Al-Kandari and Musaad Al-Roumi

***Abstract*—**Fish production systems involve regular handling of the cultured animals for sorting, transportation, tagging and vaccination. The use of anesthetics to minimize the handling harmful effect is a regular procedure. Clove oil has been used in aquaculture widely worldwide. For the organic aquaculture concept, Thyme oil has been tested and proven to be an effective alternative for use as a fish anesthetic. The optimum anesthetic dose for two important maricultured fish species, bluefin bream (*Sparidentax hasta*) and yellow fin bream (*Acantho pagruslatus*) of thyme oil, AQUI-S, clove oil and quinaldine was investigated for its antibacterial. In vivo studies indicated that both the fish species had highly reduced bacterial load after the treatments and the in vitro antibacterial activity of the thyme oil was superior to that of the other treatments. A patent with US No. 13/568,235 has been approved and will be published on 13/02/2014.

***Keywords*—**Clove, Thyme, quinaldine, anesthetic, antibacterial activity,

I. Introduction

HANDLING fish frequently in aquaculture activates requires using anesthetics. Though clove oil has been used in many aquaculture operations world over, its use has not been recommended. The US FDA has determined that eugenol is not generally recognized as Safe (GRAS) as a fish anesthetic. The chemicals used as anesthetics in fish have potential side effects that have not been investigated thoroughly on both the users and the fish. Quinaldine (2-methylquinoline) is one of the most widely used aesthetics that is being used by marine biologists, but there are some questions about its safety because of reported associations between quinaldine and thyroid abnormalities in humans and mice [1]. The only fish anesthetic approved by the U.S. Food and Drug Administration (US FDA) is 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222), but there are limitations in using MS-222 in the field which is a withdrawal period of 21 days before the treated fish can be consumed by humans [2, 3]. The choice of anaesthetics for field studies generally depends on several considerations (1) availability; (2) cost-effectiveness; (3) ease of use; (4) nature of the study; (5) allow for the immediate release of the fish into the food chain, (6) allow a swift induction of and recovery from anaesthesia, and (7) not excessively disturb the physiological balance of the fish, which would reduce its chances of survival upon release and (8) safety for the user [4].

Thyme oil of is an important commercial product obtained by distillation of fresh leaves and flowering tops of T. vulgaris. Its extract is extensively used in processed food and pharmaceutical industry. The Greeks used thyme as an antiseptic. Thyme extract is used in food systems to prevent the growth of food-borne bacteria and extend the shelf life of processed foods [5]. Thyme extract has been proven to possess antibacterial, antiparasitic and anesthetic effect [6].

This work has been conducted to investigate the antibacterial effect of the anesthetic dose of the thyme extract product and compare with that of three other commonly used anesthetics. A patent with US No. 13/568,235 has been approved and will be published on 13/02/2014.

II. Methodology and Work Plan

A. Experimental Fish and Anesthesia Optimization

About 300 fish each of yellow fin bream (*Acantho pagruslatus*) and blue fin bream (*Sparidentax hasta*) cultured and grown at Kuwait Institute for Scientific Research (KISR) facilities were used in this study. The fish were stocked in one ton tanks for two weeks for acclimatization. They were fed and maintained as per the existing husbandry practices at MFD. Temperature, salinity and dissolved oxygen were recorded daily during the experimental period. The anesthetic doses tested using clove oil, thyme extract, AQUI-S and quinaldine were 20, 40 and 60 ppm exposed up to 10 min, respectively. These dose levels were selected based on results of previously conducted experiment [7]. All the anesthetics were prepared as aqueous suspension in absolute alcohol at

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1:4 (anesthetic: alcohol) ratio for the essential oils were not soluble in water. The control group was immersed in an aqueous suspension of PBS-alcohol mix (1:4). Twenty fish in each dose of an anesthetic were treated to record sleep time, recovery time and mortalities for arriving at minimal anesthetic dose. All the anesthetics tested at an exposure time of 10 min.

B. Antibacterial Properties of Anesthesia

Once the fish anesthetized by the optimized doses for each anesthetic, surface swabs were taken from an approximate skin area of 4 cm2 from the fish. The swab was vortexed in sterile PBS for the enumeration of viable bacterial counts. The inactivation percentage was obtained by comparing the bacterial counts on the control group of fish with that of the anesthetic treated fish. The surface swab suspension was serially diluted and plated onto brain heart infusion (BHI with 15% NaCl) agar and thiosulphate citrate bile salt sucrose (TCBS) agar. Bactericidal activity of anesthetic doses was calculated using the control bacterial counts.

Four species of standard (ATCC) bacterial isolates (*Vibrio alginolyticus* -19108; *V. harveyii* – 35084; *V. parahaemolyticus* – 27519 and *Streptococcus agalactiae* - 27956) were procured. A 107 CFU/ml bacterial suspension of respective bacteria was obtained after 24-h growth (at 35^{o}C) in brain heart infusion broth. The bacterial suspensions were subjected to different concentrations of the anesthetics (20, 40 and 60 ppm) in 1.5 ml microfuge tubes with a fixed exposure time of 10 min at room temperature (24^{o}C). The serial dilutions were enumerated using spread plate method of plate count. The inactivation percentage was calculated using bacterial counts in the control (1:4 suspension of PBS:alcohol) for comparison.

III. Statistical Analysis

All results were subjected to Analysis of variance (ANOVA) for confirming the effects of thyme oil extract on different bacteria.

IV. Results and Discussion

Thyme oil extract among all anesthetics in blue fin bream (*S. hasta*) and yellow fin bream (*A. latus*) showed a distinct antibacterial activity. Thyme oil extract exhibited the best effect in reducing the surface bacterial load in fish compared to the control groups. Data of the in-vivo studies on the reduction of total bacterial load and the total Vibrio load on anesthetic treated *S. hasta* and *A. latus* is depicted in Fig 1 and Fig 2, respectively. The In vitro studies indicated over 90% reduction of all the bacterial species exposed to all concentrations of the essential oils (Fig 3) treated fish with thyme oil producing almost complete elimination of Vibrio from the surface swabs.

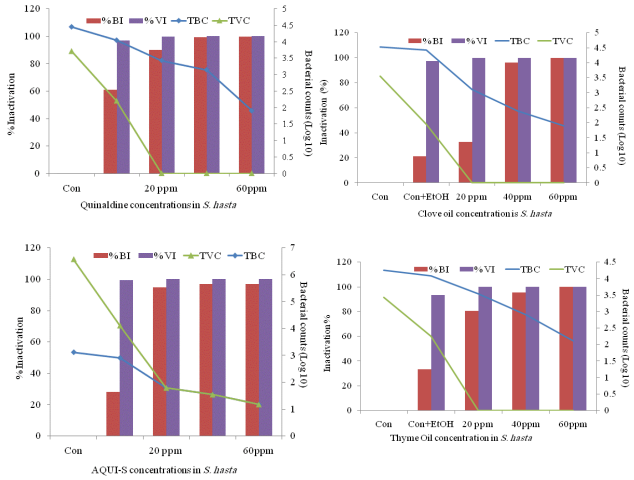

Fig.1 Surface swab from S. hasta for total bacterial and vibrio (log10) counts (TBC & TVC) and their percent inactivation after treatment.

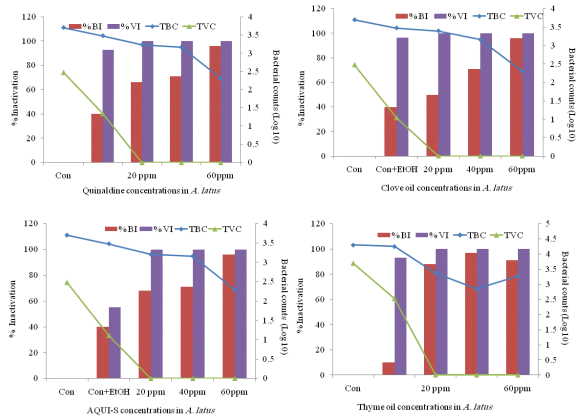

Fig.2 Surface swab from A. latus for total bacterial and vibrio (log10) counts (TBC & TVC) and their percent inactivation after treatment

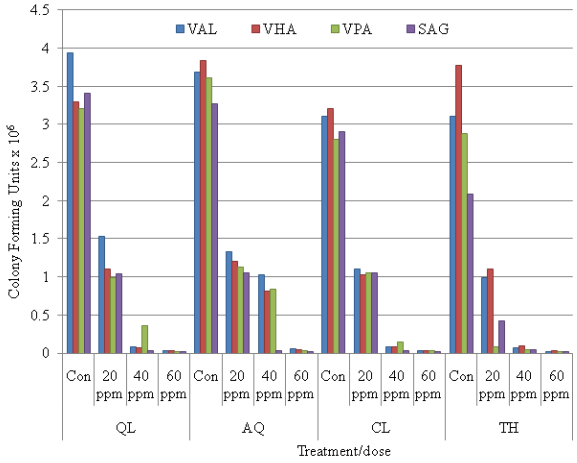

Fig.3 Antibacterial activity of different anesthetics on standard (ATCC) bacterial isolates.

Similar results on the antibacterial qualities of thyme oil have been reported earlier. Thyme oil extract as an effective antibacterial agent has been proved in the food preservation

and storage studies [5]. Thyme essential oil exhibited antibacterial activities against 25 microorganisms [8]; also, it showed inhibition of *Shigella* sp. [9] and also bactericidal effects [9, 10, and 11]. Thyme oil can also be used as antimicrobial coating on the shelf life of the precooked shrimp, *Penaeus* sp. [12]. Thyme oil was found to be very effective with a lowest minimum inhibitory concentration (MIC) of 1.25% (v/v) against *Bacillus* sp., *Listeria monocytogenes*, *E. coli* and *Klebsiella* sp. Amongst the fungi, *Rhizomucor* sp. was found to be highly sensitive to the oil. Thyme essential oil exhibited antibacterial activities against 25 microorganisms [7]; also, it showed inhibition of *Shigella* sp. [8] and general bactericidal effect [9, 13, 10, and 11].

Thyme oil extract has been proven to possess a high antibacterial activity of against many marine *Vibrios* and a Gram positive fish pathogen (*Streptococcus agalactiae*) in [6]. Complete bactericidal activity was achieved with thyme oil extract in a majority of bacterial species tested and with all concentrations of the EOs. *V. alginolyticus*, *V. anguillarum*, *V. parahaemolyticus* and *V. vulnificus* were completely killed at all levels tested with thyme oil [6 and 7] These observations suggest superior bactericidal and antiparasitic activity of thyme oil thus providing a very effective alternative anesthetic that can reduce the bacterial load on fish in addition to serving as an anesthetic.

V. Conclusion

Thyme oil extract possess antibacterial effect which make it superior to the other anesthetics and that make it very useful to be used in future application as anesthetic as well as antibacterial agent. Further studies are required to confirm this effect against different fish species.

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