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ORIGINAL ARTICLE

Antibacterial and immunity enhancement properties of anaesthetic doses of thyme (*Thymus vulgaris*) oil and three other anaesthetics in *Sparidentax hasta* and *Acanthopagrus latus*

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Abstract An effective alternative was discovered in the form of thyme oil for use as a fish anaesthetic (patent pending approval). The thyme oil along with a common aquaculture-grade commercial anaesthetic (AQUI-S), clove oil and quinaldine were investigated for their antimicrobial properties and its effect on the immune parameters of two important maricultured fish species, bluefin bream (*Sparidentax hasta*) and yellowfin bream (*Acanthopagrus latus*). *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 of the thyme oil was superior to that of the other treatments. The effects of anaesthetic dose of thyme oil, clove oil, quinaldine and AQUI-S were evaluated and compared. The reduction in the total viable vibrio counts in the anesthetized fish indicated that the vibrio were sensitive to the thyme oil. Also thyme oil produced higher non-specific immune enhancements.

1. Introduction

Aquaculture operations under intensive production systems require frequent handling of the fish of different sizes, especially during transportation, for stocking the fingerlings into production ponds and during fish breeding operations. Apart from this the fish are handled for regular observations and for the treatment, if any. 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 Recognised as Safe (GRAS) as a fish anaesthetic. Aquaculture, in the present day context, involves handling of cultured fish at different stages in the production network. Reduction of struggle during handling and trauma is the main reason for the use of anaesthetics in aquaculture. The chemicals used as anaesthetics in fish have generally been developed for purposes other than their use as anaesthetics. As a result, the potential side effects of these chemicals on users have not

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been investigated thoroughly. For example, Quinaldine (2-methylquinoline) is one of the most widely used aesthetics that is being used by marine biologists and by the aquaculture group of researchers at the Mariculture and Fisheries Department of Kuwait Institute for Scientific Research. However, there are some questions about its safety because of reported associations between quinaldine and thyroid abnormalities in humans and mice (Munday and Wilson, 1997). Another important anaesthetic used in fisheries is 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222). There are limitations in using MS-222 in the field as the US Food and Drug Administration (US FDA) requires that fish exposed to MS-222 must have a minimum of 21 day withdrawal period before they can be consumed by humans (Bernstein et al., 1997; Waterstrat, 1999). Though the anaesthetic of plant origin, the clove oil, has been used for this purpose, the US FDA has determined that eugenol is not Generally Recognised as Safe (GRAS).

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, (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. The use of thyme oil in human medicine has been approved by the FDA.

Thyme (*Thymus vulgaris*) oil is being used traditionally to treat indigestion, to promote menstruation, and for the control of fever. The essential oil of thyme is made up of 20–55% thymol, a powerful antiseptic for both internal and external use. Thyme oil is employed as local anaesthetic in human medicine, and before modern antibiotics were developed, it was used to medicate bandages. Oil of thyme is the important commercial product obtained by distillation of fresh leaves and flowering tops of *T. vulgaris*. It is extensively used in processed food. The Greeks used thyme as an antiseptic. Thyme can be used in food systems to prevent the growth of food-borne bacteria and extend the shelf life of processed foods (Baydar et al., 2004).

While working on the anti parasitic effects of some essential oils in fish (Al-Yaqout and Azad, 2010), it was noticed that the thyme oil possessed an excellent additional property of an anaesthetic. Looking at the nature of the anaesthetic and its properties, we attempted to explore the qualities of antibacterial and immune enhancement properties of thyme oil as an anaesthetic and compare with that of three other commonly used anaesthetics. This property has a direct bearing on higher survival of the fish upon release into its natural environment or aquaculture rearing facility. Thus, the investigations were conducted to evaluate the antibacterial and immune enhancement properties of thyme oil as an anaesthetic and thereby suggest measures to reduce subsequent infections after handling.

2. Materials and methods

2.1. Fish and anaesthesia optimisation

About 300 fish each of yellowfin bream (*Acanthopagrus latus*; 10.9–13.5 g) and bluefin bream (*Sparidentax hasta*; 9.9–11.7 g) were used in this study. All the fish were stocked in one ton tanks for two weeks for acclimatisation. Temperature, salinity and dissolved oxygen were recorded daily during the experimental period. The anaesthetic doses tested using clove oil, thyme oil, AQUI-S (a commercial product, AQUI-S, New Zealand Ltd.) and quinaldine were 20, 40 and 60 ppm exposed up to 10 min, respectively. These dose levels were selected based on our previous experience. Clove oil, thyme oil and quinaldine were procured from Sigma Aldrich, USA and the AQUI-S was purchased from AQUI-S New Zealand Ltd. Lower Hutt, New Zealand. All the anaesthetics prepared as aqueous suspension in absolute alcohol at 1:4 (anaesthetic: 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 anaesthetic were treated to record sleep time, recovery time and mortalities for arriving at minimal anaesthetic dose. All the anaesthetics were tested at an exposure time of 10 min. A treatment with 20 ppm of different anaesthetics was carried out until the induction of deep sleep in 10 fish in each treatment. Blood from these fish was used in the assay of immunity parameters.

2.2. Antibacterial properties of anaesthesia

2.2.1. In situ bactericidal assay

After the fish anaesthetised by the optimised doses for each anaesthetic, surface swabs were taken from an approximate

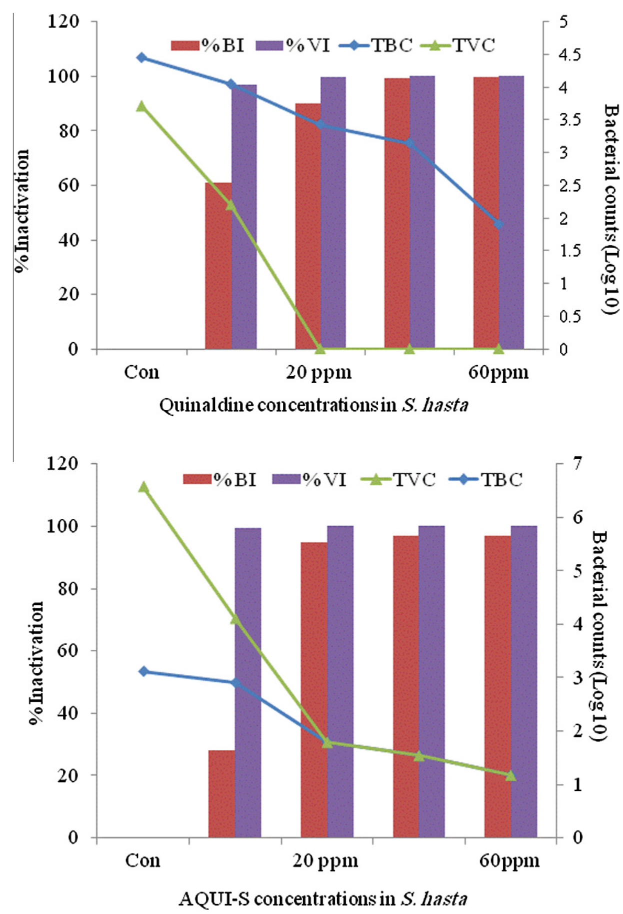

Figure 1a Surface swab from *S. hasta* for total bacterial and vibrio ($\log_{10}$) counts (TBC and TVC) and their percent inactivation after treatment with quinaldine (top) and AQUI-S (bottom).